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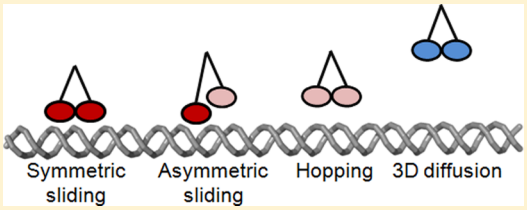
Asymmetric DNA-Search Dynamics by Symmetric Dimeric Proteins

Netaly Khazanov, Amir Marcovitz, and Yaakov Levy*

Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel

Supporting Information

ABSTRACT: We focus on dimeric DNA-binding proteins from two well-studied families: orthodox type II restriction endonucleases (REs) and transcription factors (TFs). Interactions of the protein's recognition sites with the DNA and, particularly, the contribution of each of the monomers to one-dimensional (1D) sliding along nonspecific DNA were studied using computational tools. Coarse-grained molecular dynamics simulations of DNA scanning by various TFs and REs provide insights into how the symmetry of a homodimer can be broken while they nonspecifically interact with DNA. The characteristics of protein sliding along DNA, such as the average sliding length, partitioning between 1D and 3D search, and the one-dimensional diffusion coefficient D_1, strongly depend on the salt concentration, which in turn affects the probability of the two monomers adopting a cooperative symmetric sliding mechanism. Indeed, we demonstrate that maximal DNA search efficiency is achieved when the protein adopts an asymmetric search mode in which one monomer slides while its partner hops. We find that proteins classified as TFs have a higher affinity for the DNA, longer sliding lengths, and an increased probability of symmetric sliding in comparison with REs. Moreover, TFs can perform their biological function over a much wider range of salt concentrations than REs. Our results demonstrate that the different biological functions of DNA-binding proteins are related to the different nonspecific DNA search mechanisms they adopt.



The interaction of proteins with their specific target sites on DNA is pivotal to many cellular processes. In addition to the requirement for high affinity, the protein needs to find these target sites rapidly when searching alternative nonspecific sites along the DNA. The inherent complexity of this search process, due to the large number of competing binding sites, is resolved, at least partially, by searching the DNA using both one- and three-dimensional (1D and 3D, respectively) search modes whose combination may result in facilitated diffusion.

The 1D search of DNA comprises both sliding and hopping dynamics. During sliding, the protein remains attached to DNA mostly by electrostatic interactions and traces the helical path of the DNA inside the major groove in a stochastic manner.[1−4] Sliding might be interrupted by hopping events (short-range microscopic jumps),[5] where the protein is still attracted by the DNA but more weakly than in sliding. Whereas the protein performs a rotation-coupled translation motion during sliding periods, in hopping events the protein is less committed to interact with the DNA major groove and there is therefore less friction in hopping than in sliding. Hopping can thus contribute to a faster search. DNA-binding proteins utilize two additional mechanisms that contribute to searching via facilitated diffusion: 3D diffusion and intersegmental transfer. In the former, the protein rebinds to a new site on the DNA after pure 3D diffusion in the bulk solution,[6−11] and in the latter it transfers to a distant segment through a looped DNA region.[12]

A major factor that governs the relative usage of the different search mechanisms is the ionic strength in the cell, as it directly affects the electrostatic interactions that dominate nonspecific protein−DNA interactions. The rate at which a DNA-binding protein finds its site can also be controlled by the molecular features of the proteins.[13−16] We showed, for example, that the existence of a disordered tail can increase the nonspecific affinity and the speed of linear diffusion.[12,17,18] Multidomain proteins, in which the constituent domains have different affinities to the DNA, can also promote a faster search.[19] The contribution of each mechanism (sliding, hopping, 3D diffusion, and intersegmental transfer) to the facilitated diffusion process may therefore differ from one protein to another, and these differences may be linked to their biological functions.[20]

In this study, we explore the mechanism of the DNA search conducted by dimeric DNA-binding proteins using coarse-grained molecular dynamics simulations in which the protein is flexible and dynamics, but the DNA is kept in the canonical B-form conformation. Many DNA-binding proteins bind their specific DNA sequences either as heterodimers or as homodimers. Dimeric proteins consisting of a symmetric assembly of two identical or similar subunits are ubiquitous in many cellular processes[21] as well as in gene regulatory activities. Beyond structural and functional advantages, such as higher stability and specificity, dimeric DNA-binding proteins expand the opportunities for regulation, for example, by generating a wide repertoire of DNA-binding specificities from a relatively limited number of genetically encoded proteins.[22,23] How the homodimers perform nonspecific searches for a specific DNA-binding site and what the contribution of each monomer is to the 1D sliding process

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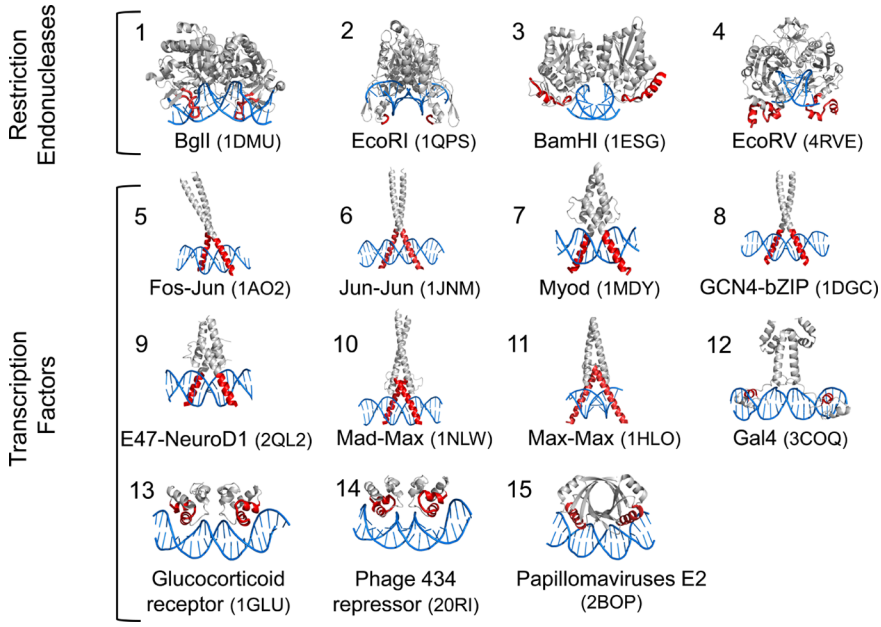

Figure 1. Dimeric DNA-binding proteins chosen for this study. Restriction endonucleases (REs, 1−4): BgII, *Eco*RI, *Bam*HI, and EcoRV. Transcription factors (TFs, 5−15): leucine zippers Fos-Jun and Jun-Jun; helix−loop−helix domains Myod, GCN4-bZip, E47-NeuroD1, Mad-Max, Max-Max, and Gal4; the glucocorticoid receptor; the phage 434 repressor transcription factor; and the papillomavirus-1 E2 DNA-binding domain transcription factor. All the transcription factors are eukaryotic except the phage 434 repressor and the papillomavirus-1 E2 DNA-binding domain, which are prokaryotic and viral, respectively. The PDB entry of each dimeric RE or TF appears in parentheses.

are challenging questions. To answer them, we investigated the nonspecific protein−DNA interactions and search mechanisms of several dimeric protein transcription factors (TFs) and dimeric proteins from the family of type II restriction endonucleases (REs).

TFs bind specific genome sites and either activate the recruitment of additional components necessary for the transcription of the associated gene or repress the expression of the gene.[24] Several studies of TF families, such as bHLHZip, bZIP, NF-κB, and STATs, have shown that many TFs form homotypic dimers[25] and that the DNA-binding domain is the most conserved portion of the protein in all families. The dimerization is likely to occur via an intermediate step where the two protein subunits first assemble individually on nonspecific DNA.[26−28] It has also been demonstrated experimentally[29] and computationally[30] that DNA may enhance the rate at which the two subunits assemble and fold. The representative TFs chosen for this study are several systems representing different binding motifs, such as leucine zippers, helix−loop−helix, and Zn2/Cys6 DNA-binding domains, that originated from a variety of species ranging from phage 434 to human (Figure 1).

REs are prokaryotic enzymes that protect the cell by recognizing and cleaving an invasive DNA sequence.[31,32] They belong to a restriction-modification system characterized by endonuclease and methyltransferase activity. Methylated host DNA is protected from cleavage by REs, while the unmethylated foreign phage genome is targeted for enzyme activity.[33] REs are classified into three types based on their subunit composition, enzyme cofactor requirements, the nature of their target DNA sequences, and the position of their DNA cleavage site relative to their target sequence.[34,35] In the present study, we investigated enzymes from the 3000-member family of type II REs,[36,37] with a particular focus on homodimeric orthodox type II enzymes that recognize relatively short (4−8 bp) double-stranded DNA (dsDNA) palindromic sequences.[38−41] Crystal structures of many REs indicate the presence of a core whose structure is highly conserved. This core carries out most of the catalytic function of the REs, which implies that type II REs share a common ancestor.[42] Mostly on the basis of their structural and functional similarities, type II REs are divided into the *Eco*RI-like family, which binds the DNA major groove (*Eco*RI, *Bam*HI) and produces sticky ends in the cleavage (5′ or 3′ overhangs), and the EcoRV family, which recognizes DNA through the minor groove and typically produces blunt ends in the cleavage.[41] The REs have been used in several cases to study the characteristics of facilitated diffusion. Sliding has also been demonstrated as a significant search mechanism for the *Eco*RI,[43,44] *Bam*HI,[45] and EcoRV[46,47] enzymes. For EcoRV, hopping and 3D search of nonspecific DNA were also reported.[48]

Here, we employed computational tools to investigate in detail the nonspecific interactions of 15 dimeric DNA-binding proteins, REs and TFs, with the goal of understanding the role that each monomer plays during the search on DNA (Figure 2). While interacting with DNA as a dimeric protein might be advantageous for higher affinity and selectivity,[49,50] it is questionable whether the dimeric state offers any advantages in terms of search speed. A protein composed of two identical subunits necessarily has twice the protein−DNA interface. Consequently, linear diffusion might be slower. We ask whether it is more efficient to search as a dimer or as a monomer. We demonstrate that the symmetry and cooperative nature of the sliding mechanism are highly dependent on the salt concentration. Interestingly, optimal search efficiency is achieved when the asymmetric search mode, in which one monomer slides in close proximity to the DNA while its partner moves by hopping, is preferred for 1D diffusion (Figure 2). We also highlight differences in the characteristics of the nonspecific interactions of TFs and REs with DNA: the former

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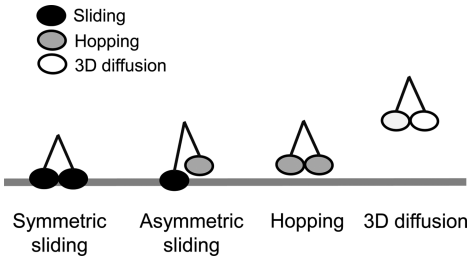

Figure 2. Schematic illustration of the mechanisms by which a homodimeric protein may search DNA. Asymmetry in the interaction between the protein and DNA can result in one-dimensional diffusion in which one monomer scans DNA using the sliding mode while the other monomer hops.

have a higher affinity for DNA and are more likely to adopt symmetric sliding (i.e., by both monomers) and to slide for longer distances along DNA in comparison with REs.

MATERIALS AND METHODS

We employed a coarse-grained representation of protein−DNA systems to allow simulations of protein translocations along DNA over relatively long biological time scales.[3,51,52] We studied the interactions of 11 TFs and 4 REs (listed in Figure 1) with nonspecific DNA. The TF and RE proteins were modeled at the resolution of a Cα atom, where each amino acid was represented by a single bead located at the position of the Cα atom. We employed the native topology based model (Gō-model), in which native contacts interactions are represented by Lennard-Jones potential and nonnative interactions are excluded, to account for the dynamics of the protein.[53,54] The simulations were carried out at low temperatures and capture protein flexibility, but protein folding−unfolding events were not addressed here (the temperature was $0.6T_F$, where T_F is the folding temperature at which the folded and unfolded ensembles are equally populated). Nevertheless, the temperature was sufficiently high to allow internal dynamics of the proteins and relative dynamics between the protein subunits. We used a 100 bp dsDNA molecule in its canonical B-form that was held rigid and static throughout the simulation and was aligned with the *Z*-axis. The protein and the DNA were placed in a box with dimensions of 150 Å × 150 Å × 350 Å. Each nucleotide in the DNA was represented by three beads positioned at the corresponding centers of the phosphate, sugar, and base groups. The modeling of the DNA as a rigid molecule in a B-form conformation is based on many observations in which conformational changes of the DNA are linked to specific recognition. Although there are some sequence-nonspecific architectural proteins, such as heat unstable (HU) and high-mobility group B (HMGB) proteins,[55,56] that are capable of transient binding and kinking of DNA, we assume that TFs and REs interact with DNA while the DNA does not undergo severe conformational changes. This assumption is supported by various studies that indicate that the DNA remains in B-DNA form when it nonspecifically interacts with proteins and that conformational changes are associated with specific recognition. Nonetheless, the DNA may still fluctuate around the B-DNA conformations which may result with local and transient changes in the grooves geometric parameters. The effect of DNA dynamics on the molecular mechanism of DNA search is not yet quantified and is neglected in the current study.

Beads corresponding to the phosphate group were assigned a negative point charge of −1, as were the protein beads that correspond to the negatively charged amino acids (Asp and Glu). Accordingly, protein beads representing positively charged amino acids (Lys and Arg) were assigned a charge of +1. Electrostatic interactions were introduced into the simulation through the Debye−Hückel potential,[3,57] which allowed us to introduce salt concentration effects ranging from 0.01 to 0.12 M. The dielectric constant in the simulation was 40. No additional protein−DNA interactions were modeled except for electrostatic and excluded volume effects; therefore, our model addressed solely nonspecific protein−DNA interactions. While the Debye−Hückel model is powerful in introducing the salt effect of screening electrostatic interactions into the Coulomb potential, one should be aware of its approximations. The model is valid for relatively dilute conditions, as it approximates that the potential energy of an ion is determined by pairwise interactions with other neighboring ions. The detailed effects of higher salt concentrations and of ion condensation on DNA have to be studied using the nonlinearized Poisson−Boltzmann equation as well with atomistic simulations that can elucidate the dynamics of the ionic layer during sliding on the DNA.

Since the model is coarse-grained, the distances between the charged beads of the protein and the charged DNA beads are longer compared to fully atomistic models (the charges are placed at the phosphate and C_α beads instead of their actual atoms). Consequently, the salt concentrations reported in this paper using the coarse-grained model (10−50 mM) are smaller than typical physiological salt conditions (∼100−150 mM). The range for the salt condition selected in this work is however a measure of strength within the model that successfully recapitulates many important characteristics of protein search modes on DNA.[3,12,17,19,52]

To achieve a comprehensive sampling of each protein system, we performed three Langevin molecular dynamics simulations of 10^8 time steps for each protein variant at each salt concentration. Each simulation frame was structurally classified as belonging to one of three search mechanisms: sliding, hopping, or 3D diffusion. A snapshot of the simulation was defined as taking part in sliding if it met three criteria as defined in ref 3. First, at least 65% of the recognition region of the monomer as part of the dimer was in contact with the major groove. Second, it maintained a similar orientation to that found during specific DNA binding. Third, the distance of the center of mass of the recognition region from the DNA was longer than that in the crystal structure by at least 10 Å (in the case of a helical recognition site) or 14 Å (in the case of a looped recognition site). Sliding of the dimer can be performed in a symmetric or asymmetric fashion, depending on the search mode of each monomer that comprises the dimer. In hopping, the protein remains in close proximity to the DNA but is not restricted to any particular orientation. For 3D diffusion, the protein is essentially dissociated from the DNA, and the electrostatic energy between the protein and DNA is negligible.[3,57] To quantify the efficiency of DNA search by a protein, we employed the position-probed measure.[3,17,18] This measure indicates the number of new DNA sites (base pairs) visited by the protein during sliding throughout the simulation. We used the Langevin equation to simulate the system (with γ = 0.01). The 1D diffusion coefficient D_1 was evaluated from the mean-square displacement profiles obtained from the trajectory of the protein center of mass propagating along the *Z*-axis (i.e.,

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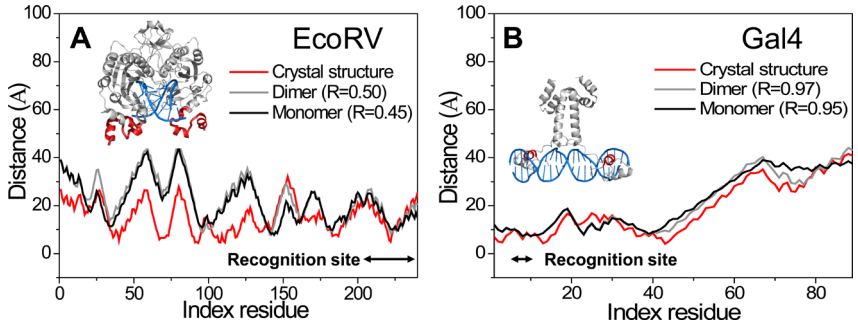

Figure 3. Structural similarity between nonspecific and specific binding to DNA. Average distances of each of the protein residues to the closest DNA atom during the simulation as the protein searches as a dimer (gray line) or as two individual subunits (black line). The distance profiles, calculated at a salt concentration of 0.01 M, are compared with the distance profiles in the crystal structure (red line) for (A) EcoRV and (B) Gal4. Correlation coefficients indicate the similarity of each profile to the profile in the crystal structure.

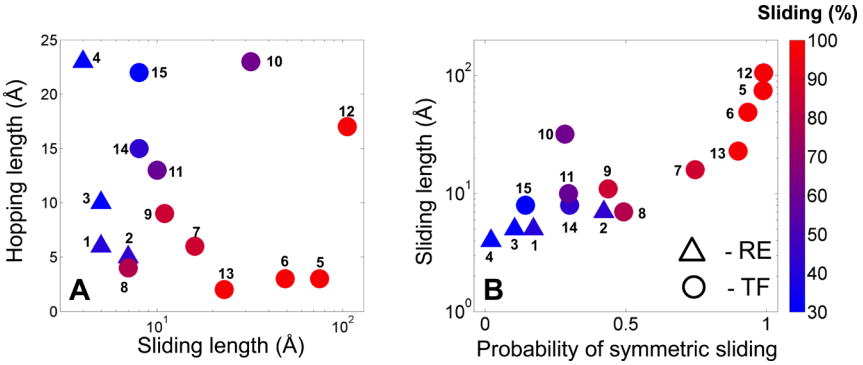

Figure 4. Characteristics of sliding performed by dimeric proteins. (A) Mean length of a sliding event against the mean length of a hopping event calculated at a salt concentration of 0.03 M for each restriction endonuclease (RE; triangles) and transcription factor (TF; circles). Color coding indicates the amount of sliding performed as a fraction of the total search time. Most TFs prefer the sliding mode, which they adopt for >50% of their search time, while hopping is the dominant mechanism for REs. (B) The more a dimer slides, the higher the probability that it will slide symmetrically and the longer the distance traveled in each sliding event. RE dimers perform sliding over shorter distance ranges in comparison with TFs, and sliding is a less dominant search mechanism for REs. TFs perform more sliding over longer ranges, indicating that they have a higher affinity for DNA. Dimers that utilize symmetric sliding perform sliding over longer distances and adopt the sliding mechanism for a larger proportion of their total search time than dimers that utilize other search mechanisms. The number next to each symbol (a circle or a triangle) corresponds to the index number of each protein as appears in Figure 1.

the axis with which the DNA is aligned). Additional methodological details (including assumptions and simplifications of the model) used to study nonspecific protein–DNA interactions and the dynamics of proteins when searching the target site can be found in previous publications.[3,12,17,19,51,57]

RESULTS AND DISCUSSION

Characterization of Protein Search on DNA. Here, we studied dimeric REs and TF DNA-binding proteins that represent a wide repertoire of DNA-binding domains and origins (Figure 1). The REs group consisted of four representatives: EcoRV,[58] *Eco*RI,[59] BglI,[60] and *Bam*HI.[61] The TF dimeric systems included eukaryotic representatives: leucine zippers Fos-Jun[62] and Jun-Jun (PDB ID 1JNM); basic helix–loop–helix DNA-binding domain proteins E47-NeuroD1,[63] Myod,[64] Mad-Max,[65] and Max-Max,[66] as well as the yeast Gal4,[67] and a glucocorticoid receptor.[68] In addition, a prokaryotic and a viral TF were selected: the phage 434 repressor[69] and the papillomavirus-1 E2 DNA-binding domain,[70] respectively. In the complex formed by the E2 DNA-binding domain with DNA, the DNA is bent to enable the recognition helix to approach binding at the major groove. However, since we focus here on features of nonspecific DNA binding, we used the structure of the free papillomaviral E2 protein (PDB ID 1DBD), which deviates in its tertiary structure from the protein in the DNA-bound state.[71] For the RE *Bam*HI, we used the crystal structure of the protein bound to nonspecific DNA (PDB ID, 1ESG),[61] in which an α-helix is formed at the C-termini of the protein.

We performed coarse-grained molecular dynamic simulations for each protein to study the nature of its search along a 100 bp dsDNA that is held static throughout the simulation at a low temperature that maintains the protein in a folded state. A wide range of salt concentrations (0.01–0.12 M) was introduced into the model through the Debye–Hückel potential. Figure 3 presents the average distance of each residue from the closest DNA atom for EcoRV (Figure 3a) and for the transcription factor Gal4 (Figure 3b). We compared the distances in the crystal structure with the distances calculated under a low salt concentration of 0.01 M as the protein slides as an intact dimer and as two isolated monomers (additional proteins are shown in Figure S1). The profile of distances for Gal4 during sliding (both as a dimer and as two individual monomers) strongly resembles the profile observed in the crystal structure, whereas the distance profiles for the EcoRV enzyme deviate more significantly from the distance profile in the specific complex. However, the recognition sites of the proteins (indicated by the arrows in Figure 3) maintain a very similar profile to those in

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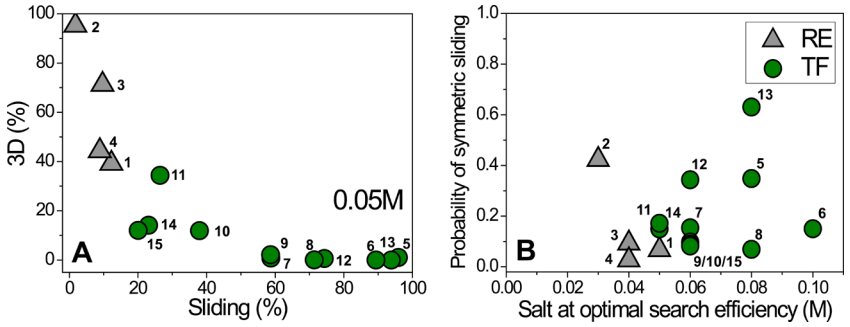

Figure 5. Characteristics of the search performed by dimeric proteins. (A) The average usage of sliding (either symmetric or asymmetric sliding) and 3D diffusion for the studied dimeric proteins at a salt concentration of 0.05 M. Most transcription factors (TFs; circles) prefer the sliding mode, which they adopt for >50% of their search time, while hopping is the dominant mechanism for restriction endonucleases (REs; triangles). (B) At the salt concentration at which maximal search efficiency is achieved, sliding is less symmetric than at a lower salt concentration (e.g., 0.03 M) at which most TFs tend to interact with DNA using the symmetric sliding mode (see Figure 4B). This implies that symmetric sliding by the two DNA-binding domains reduces the efficiency of 1D movement by a dimer along the DNA. The number next to each symbol (a circle or a triangle) corresponds to the index number of each protein as appears in Figure 1.

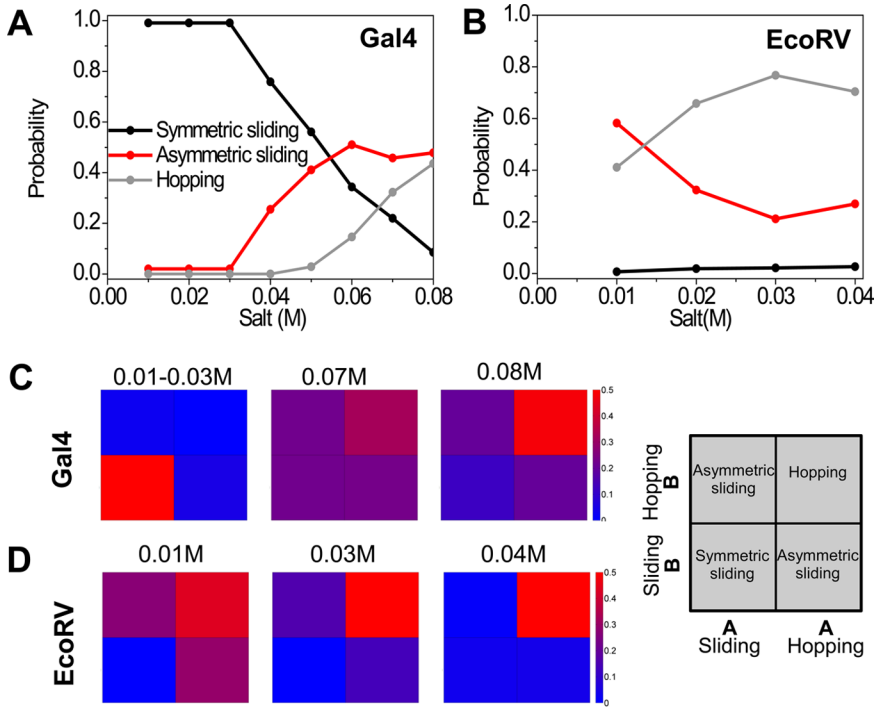

Figure 6. Symmetric and asymmetric sliding by the two DNA-binding domains of (A) a Gal4 dimer and (B) an EcoRV dimer calculated under varying salt conditions. For each dimer, the probabilities for symmetric sliding, asymmetric sliding (one subunit slides while its partner subunit hops), and symmetric hopping are indicated by black, red, and dashed blue lines, respectively. The sum of these three probabilities add to unity. The Gal4 dimer adopts symmetric sliding at low salt concentrations and gradually switches to asymmetric sliding and finally simultaneous hopping by both subunits as the salt concentration increases. For the EcoRV dimer, symmetric hopping dominates with occasional DNA probing by one of the subunits through the sliding mode under low salt conditions. Matrices of the adoption of sliding/sliding (lower left corner), sliding/hopping (upper left and lower right), and hopping/hopping (upper right) search modes under various salt conditions for (C) Gal4 and (D) EcoRV. The colored bar to the right of each matrix indicates the dimer population of each search mode and ranges from blue (0%) to red (50% and higher).

the crystal structure. The result for the restriction enzyme is supported by recent experimental observations suggesting that the 1D translocations of the protein along DNA are orders of magnitude faster than expected from a pure sliding motion in which the protein remains in close proximity to the DNA and traces the helical DNA path. Such a rapid 1D movement involves, in addition to pure sliding, more hopping events and jumps along the DNA and was observed experimentally to occur in several REs.[16,48,72,73] The high usage of hopping and jumping was illustrated to be linked with the ability of some REs to bypass obstacles located on the DNA.[74]

Several studies have suggested that many TFs originally functioned as monomers.[75,76] This proposition has been confirmed for several systems by experiments that show the ability of individual TF subunits to interact with DNA both specifically and nonspecifically.[26,27,29,77−80] Our results indeed show that, in most of the systems, individual monomers are able to search DNA using a combination of the three mechanisms—sliding, hopping, and 3D diffusion—even though their affinity for the DNA is reduced compared with the dimer. Moreover, for most TFs, the orientation of the searching monomer and its distance from the DNA are similar

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to those observed when the search is undertaken by a dimeric protein (see Figure S1).

Figure 4a presents the relationship between the fraction of the search undertaken using the sliding mechanism and the average DNA length scanned by sliding compared with hopping for the TFs and REs studied at a 0.03 M salt concentration. As the fraction of the search performed by sliding increases, the interactions of the protein with DNA become tighter and the protein takes relatively long sliding excursions in which any hopping events are sparse. It can be observed that most TFs engage in longer-range sliding events than REs, which indicates that TFs have a higher nonspecific affinity for the DNA than REs.

The higher nonspecific affinity of TFs for DNA found in Figure 3 is supported by Figure 5a, which shows the percentage of sliding compared with 3D diffusion by TFs and REs at a higher salt concentration of 0.05 M. While most TFs maintain their sliding search mode at higher salt concentrations, REs dissociate from DNA in a significant portion of the searches when the salt concentration rises. This result suggests that these two DNA-binding protein types, which are distinguished from each other in terms of their biological roles in regulating DNA, also exhibit distinct search processes. TFs search mostly by sliding and are less sensitive to increased salt concentrations, while REs combine sliding along with a significant amount of hopping and are therefore more sensitive to changes in salt concentration.[81]

Symmetric versus Asymmetric Sliding in Dimeric DNA-Binding Proteins. The existence of heterodimeric DNA-binding domains is beneficial as it enables proteins to recognize a variety of DNA target sites.[49,50] However, many DNA-binding proteins interact with DNA as homodimers, and intuitively, one would expect the two subunits to adopt a symmetric search mechanism in which the two monomers interact identically with the DNA. Asymmetry in nonspecific protein−DNA binding has been discussed in the past in terms of the structural effects of symmetry breaking on specificity.[82,83] Here, we investigated the contribution of each monomer to the search on nonspecific DNA as the protein interacts with DNA in its dimeric form. More specifically, we addressed the search mechanism adopted by each monomer and asked whether the sliding/hopping modes adopted by two bound monomers are correlated with each other. We note that both sliding and hopping correspond to 1D diffusion on the DNA, with sliding being performed when the protein interacts more tightly with the DNA while it follows the major groove. In hopping events, however, weaker coupling between translation and rotation while scanning the DNA is expected.

Figure 6 (upper panels) shows the probability of Gal4 (Figure 6a) and EcoRV (Figure 6b) adopting symmetric and asymmetric sliding (additional proteins are shown in Figure S2). Under low salt concentrations (0.01−0.04 M), the probability for simultaneous sliding by the two monomers is very high for Gal4, indicating that the two recognition helices move along the DNA in a random walk helical motion while maintaining interactions inside the major groove of the DNA. The probability for asymmetric sliding, in which the recognition helix of one subunit slides while the other hops, increases as the salt concentration increases (>0.05 M). We note that in asymmetric sliding the protein performs helical motion along the scanned DNA which is dominated by the subunit which more tightly interacts with the major groove and slides. The matrices in the lower panel indicate the probability of the two monomers adopting sliding/sliding or hopping/hopping (i.e., symmetric sliding, see Figure 2) and hopping/sliding or sliding/hopping (i.e., asymmetric sliding) at varying salt concentrations. The increasing populations of the hopping/sliding and sliding/hopping search modes at high salt concentrations (>0.05 M) in contrast with the dominant population in the sliding/sliding mode (lower left corner of the maps) at salt concentrations of 0.01−0.03 M reflect symmetry breaking in nonspecific interactions between Gal4 and DNA as the salt concentration increases.

In contrast to the symmetry breaking pattern of nonspecific protein−DNA interactions observed in Gal4 under increasing salt concentrations, the two subunits of EcoRV perform mostly hopping at salt concentrations ranging from 0.01 to 0.04 M (Figure 6b). However, at low salt conditions of 0.01 M, one of the protein subunits occasionally samples the DNA in the sliding mode and thus approaches the DNA major groove more closely, while the partner protein subunit hops. This tendency is reflected in Figure 6d, which shows a high population in the upper-right hopping/hopping mode coupled with moderate populations in the sliding/hopping and hopping/sliding modes (lower right and upper left quadrants) at a salt concentration of 0.01 M. Utilization of the latter, asymmetrical search mode is observed to decrease with increasing salt concentrations until only the hopping search mode remains at an ionic strength of 0.04 M. Our observation for EcoRV is in agreement with a recent experimental study of the movement of the restriction enzyme on noncognate DNA which showed that EcoRV scans DNA using the hopping search mode rather than the sliding mode.[48]

We found symmetry breaking patterns with increasing salt concentration for most of the TF systems chosen for this study, while for most REs, we observed similar behavior to that observed in EcoRV (see Figure S2). These results demonstrate a difference in the search processes along DNA taken by dimeric TFs compared with REs: while at low salt concentrations, the two TF subunits probe the DNA through a helical motion in the major groove (sliding), REs preferentially scan the DNA through simultaneous subunit hopping, with one subunit occasionally and transiently sliding.

We note that our results of symmetry breaking in sliding may be valid not only for dimeric DNA-binding proteins but also for other symmetric proteins or proteins with large interface to the DNA. In all these cases, we suggest that only part of the interface will be in use in the nonspecific mode and at physiological salt concentration. With respect to the dimeric proteins reported in this study, one may view the dimeric REs as a single protein due to the limited flexibility between the constituent subunits and that they form a continuous interface with the DNA, comparing the some dimeric TFs whose subunits are quite independent and interact with DNA sequences that are spaced with DNA linkers. We speculate that the higher tendency of REs to slide asymmetrically is related to the coupling between their monomers. A concomitant sliding of the two subunits of the symmetric dimers might be restricted by the difficulty to fully form the extensive protein−DNA interface when the protein is quite rigid and the DNA is in B-DNA conformation. The formation of the entire protein−DNA interface might be easier upon formation of the specific complex that in many cases involves conformational changes of either or both the protein and the DNA. When the coupling between the dimer subunits is reduced (i.e., larger protein flexibility), they can both form tight

interaction with the DNA in its B-DNA form and therefore search the DNA via symmetric sliding.

Effects of Symmetric and Asymmetric Sliding on Protein Search along DNA. We first investigated the relationship between use of symmetric sliding and the overall search mechanism adopted by the protein. Figure 4b shows the average length of the DNA sampled by sliding against the probability of symmetric sliding occurring as well as the fraction of the search undertaken using sliding, measured at a moderate salt concentration of 0.03 M. For dimeric TFs, which preferentially use the sliding mechanism, larger use is made of symmetric sliding and the average lengths of the DNA sampled by sliding are longer than those found in RFs, in accordance with the higher affinity of TFs for DNA. REs in contrast, have a lower affinity for DNA and perform more hopping to move one-dimensionally along DNA. Consequently, they sample DNA through the sliding mode more transiently and less symmetrically than TFs.

We next asked whether symmetric or asymmetric sliding is associated with a more efficient search process. To answer this question, we sought the salt concentration at which the number of new sites visited by the protein during sliding is maximized for each system (see Materials and Methods for more details). Under each such salt condition, we calculated the probability of each protein undertaking symmetric sliding. Figure 5b shows that the symmetry of sliding is broken in most systems to achieve the maximal search efficiency. Moreover, the typical salt concentration at which maximal efficiency is achieved ranges from 0.03 to 0.1 M. Under such salt conditions, a typical DNA-binding protein would utilize more hopping (and possibly some 3D diffusion) rather than sliding.[3] We therefore propose that asymmetric sliding, in which only one subunit probes the DNA content in the major groove through a helical motion while the other one hops between nearby sites, is a plausible scenario for optimal search by dimeric DNA-binding proteins. Such a scenario for searching along nonspecific DNA has been recently observed in multidomain DNA-binding proteins.[19] By contrast, cooperative sliding by the two subunits will be less productive in terms of DNA sampling.

We measured the 1D diffusion coefficient, D_1, of an intact dimer from mean-square displacement profiles of the protein translocations along the axis of the DNA.[3] Figure 7 shows the ratio of the D_1 of an intact dimer (dimer A in Figure 7) to that of a dimer in which one subunit is neutralized (so eliminating its interactions with DNA; dimer B in Figure 7) against the probability for symmetric sliding. The D_1 for dimers that use symmetric sliding (mostly TFs) are affected dramatically by the neutralization of one of the subunits. Their overall affinity for the DNA is reduced, and the neutralized subunit no longer interferes with the random walk motion of its partner. In contrast, for proteins that utilize mostly asymmetric sliding (primarily REs), neutralization does not much affect the diffusion coefficient because the nonspecific interaction with the DNA is *a priori* determined by a single DNA-binding domain.

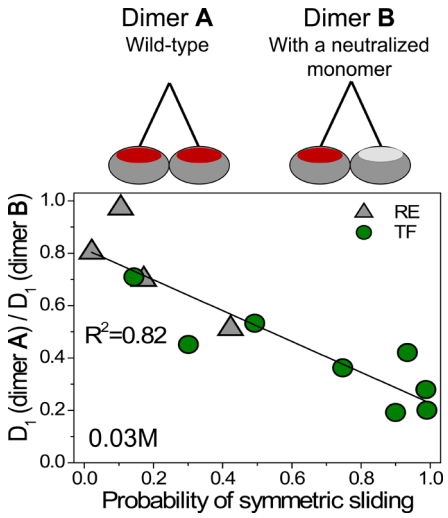

Figure 7. Effects of symmetric/asymmetric search on the one-dimensional diffusion coefficient for restriction endonucleases (REs; triangles) and transcription factors (TFs; circles) at a salt concentration of 0.03 M. Ratio of the 1D diffusion coefficient D_1 of the intact dimer to that of a dimer in which one subunit is neutralized and therefore lacks any nonspecific interactions with the DNA. Neutralization of one subunit eliminates interference by that subunit in the other subunit's ability to 1D propagate (i.e., slide) along DNA and therefore dramatically enhances sliding by the non-neutralized subunit.

SUMMARY AND CONCLUSIONS

In this paper, we addressed the interactions of dimeric TFs and REs with nonspecific DNA. Such interactions are crucial to the process of searching specific DNA sequences. In particular, we investigated whether two bound subunits of a dimeric protein perform cooperative sliding along nonspecific DNA and, if so, what the global effects of symmetric or asymmetric translocations are on the overall characteristics and efficiency of the search process. We demonstrated how the symmetry can be broken as the salt concentration increases and found distinct types of symmetries in TFs and REs. Many TFs perform cooperative sliding at low salt concentrations, whereas at higher salt concentrations, one DNA-binding domain helically slides inside the major groove while the other hops. By contrast, the REs chosen for this study propagate mostly through symmetric hopping by both subunits with transient DNA sampling through sliding by one of the subunits at a time. Moreover, as the dominance of symmetric sliding increases, the overall affinity for DNA increases, which in turn leads to longer average distances traveled by sliding, a higher fraction of sliding in the total search, and a lower 1D diffusion coefficient. Accordingly, we find that, in order to achieve maximal search efficiency on DNA, dimeric DNA-binding proteins prefer the asymmetric search mode.

Some dimeric DNA-binding proteins were found to interact with DNA via a monomeric pathway; namely, their dimerization is mediated by the DNA as they are attracted to the DNA as monomers.[26−28] The population of the monomeric pathway depends on the dissociation constant of the dimer and on the rate of binding of the monomers to the DNA. The monomeric pathway was shown mostly to TFs with relatively small dimeric interface[77] and dissociation constant in the nanomolar range.[27] Together with the observation for the potential of some TFs to initially bind DNA molecules in their monomeric form, it was reported that the DNA itself facilitates the dimerization process.[29,30] Accordingly, these TFs may still search DNA as a dimer than as two separated monomers. Obviously, the concentration of the monomeric form of homodimeric DNA-binding proteins reduces as the dissociation constant is smaller (for example, the RE *Bam*HI was found to be homodimeric in solution), thereby making the asymmetric search mode during 1D diffusion more relevant.

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Symmetry is common in protein assemblies, homodimers, and higher homooligomers and is known to confer structural and functional advantages.[84−86] However, symmetry breaking was illustrated in various assemblies composed of identical subunits[83,87] and was suggested to have important functional consequences. In particular, asymmetric binding of homodimeric proteins to DNA was observed in several protein−DNA complexes. In some cases, homodimeric proteins adopt dramatically asymmetric structures to recognize cognate DNA targets.[88] Furthermore, even when the asymmetry in the structures of homodimeric DNA-binding proteins is subtle, it changes the specificity of the interaction.[82] Our study suggests that symmetry breaking in the interactions between proteins and DNA might be useful not only for specific binding but also for nonspecific binding. Sliding by a dimeric protein in which only one monomer is situated in the major groove whereas the other hops between nearby sites reduces the friction associated with sliding because the interface between the protein and the DNA backbone is half that calculated for symmetric sliding when the two monomers interact simultaneously with the DNA.

Asymmetric sliding of dimeric DNA-binding proteins can be viewed as a means to resolve the speed−stability paradox, which indicates a conflict between the need to interact tightly with DNA to ensure stability versus the requirement to rapidly search for the target site.[6,15] The high stability of the dimers due to the larger interface they form with DNA can be decoupled from the slower search speed by adopting the asymmetric sliding mode, which is characterized by a higher D_1 than the symmetric sliding mode. We have recently concluded for other protein systems (e.g., tailed[12,18] and multidomain[19] proteins) that asymmetry in their structure or dynamics is essential for achieving both high stability and a fast search. For example, for the Egr-1 TF, which is comprised of three symmetric zinc-finger domains, it was shown using NMR and coarse-grained simulations that the first finger is much more mobile than the other two fingers, and this asymmetric dynamics facilitates the search kinetics.[89]

Our observation for EcoRV is in agreement with a recent experimental study which showed that the EcoRV performs rotation-coupled sliding (either symmetric or asymmetric) at low salt concentration on noncognate DNA[90] but uses the hopping search mode rather than the sliding mode[48] at higher salt concentration. For other DNA-binding proteins it was also shown that they can linearly diffuse on DNA using two modes (simple translation (i.e., hopping) or rotation-coupled translation).[91] The current study predicts that the D_1 of the studied REs will increase more dramatically with the increase of salt concentration than the TFs because they use more the hopping search mode. We propose that the asymmetric sliding might be probed by bimodal distribution of distances each monomer of the symmetric dimeric protein forms with the DNA.

This work, together with previous works on p53,[52,92] demonstrates that the relatively simplified model used here can usefully address various aspects of the functioning of oligomeric assemblies on DNA and how DNA affects their assembly.[30] The results presented here not only complement previous experimental studies on nonspecific interactions between DNA and TFs[27,28] or REs[46,47] but also provide new insights for further studies on the relationships between the biological function of a DNA-binding protein and the mechanism of DNA search.

■ ASSOCIATED CONTENT

Supporting Information

Average distances from each of the protein residues to the closest DNA atom during the simulation as the protein searches as a dimer or as two individual subunits (shown in Figure 3 for EcorV and Gal4) are shown in Figure S1 for all the studied dimers; the symmetric and asymmetric sliding by two DNA-binding domains of a dimer calculated under varying salt conditions (shown in Figure 6 for EcorV and Gal4) are shown in Figure S2 for all the studied dimers. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail koby.levy@weizmann.ac.il; phone 972-8-9344587 (Y.L.).

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Notes

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