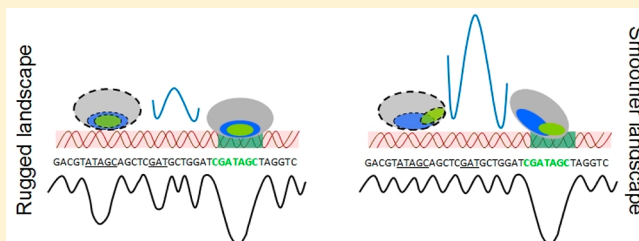


Weak Frustration Regulates Sliding and Binding Kinetics on Rugged Protein–DNA Landscapes

Amir Marcovitz and Yaakov Levy*

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

ABSTRACT: A fundamental step in gene-regulatory activities, such as repression, transcription, and recombination, is the binding of regulatory DNA-binding proteins (DBPs) to specific targets in the genome. To rapidly localize their regulatory genomic sites, DBPs reduce the dimensionality of the search space by combining three-dimensional (3D) diffusion in solution with one-dimensional (1D) sliding along DNA. However, the requirement to form a thermodynamically stable protein–DNA complex at the cognate genomic target sequence imposes a challenge on the protein because, as it navigates one-dimensionally along the genome, it may come in close contact with sites that share partial or even complete sequence similarity with the functional DNA sequence. This puzzling issue creates a conflict between two basic requirements: finding the cognate site quickly and stably binding it. Here, we structurally assessed the interface adopted by a variety of DBPs to bind DNA specifically and nonspecifically, and found that many DBPs utilize one interface to specifically recognize a DNA sequence and another to assist in propagating along the DNA through nonspecific associations. While these two interfaces overlap each other in some proteins, they present partial overlap in others and frustrate the protein–DNA interface. Using coarse-grained molecular dynamics simulations, we demonstrate that the existence of frustration in DBPs is a compromise between rapid 1D diffusion along other regions in the genome (high frustration smoothens the landscape for sliding) and rapid formation of a stable and essentially active protein–DNA complex (low frustration reduces the free energy barrier for switching between the two binding modes).



INTRODUCTION

A key component of gene regulation is the binding of regulatory proteins to specific target sequences in the genome. The gene expression process is very demanding, and requires that DNA binding proteins (DBPs) must recognize their cognate sites and distinguish them from other similar sequences that are prevalent in large genomes. Given the size of the genome and the crowded environment in which these search and binding processes take place, the remarkable efficiency with which many DBPs recognize their DNA targets presents a major puzzle. Indeed, DBPs are capable of localizing their cognate sites at a rate that far exceeds the 3D diffusion limit.¹ To explain this phenomenon, Berg and Von-Hippel proposed that the search process for a DNA target is facilitated through a combination of three-dimensional (3D) diffusion in solution and one-dimensional (1D) sliding periods in which the protein propagates along the DNA in a random-walk fashion.^{2,3}

Several structures obtained for proteins bound to semi-specific DNA sequences (i.e., with partial sequence similarity to the specific DNA target) have highlighted that nonspecific interactions are mostly dominated by electrostatic attraction between positively charged protein side chains and the negatively charged DNA backbone.^{4–6} This notion is supported by a greater dependence on salt concentration in nonspecific interactions compared with specific protein–DNA complexes.^{7,8} Accordingly, many DBPs have positively electrostatically charged patches at their surface that complement the negatively charged DNA,^{9,10} while negatively charged amino

acids are less common at protein–DNA interfaces.¹¹ In contrast to nonspecific protein–DNA association, which is dominated by electrostatic interactions, specificity in protein–DNA interactions is mostly achieved through the formation of hydrogen bonds between donors and acceptors from protein side chains and DNA bases,¹² stabilized by van der Waals and hydrophobic forces, electrostatics, and water-mediated interactions between polar groups.^{13,14}

NMR measurements of the kinetics and sliding dynamics of the HoxD9 homeodomain on DNA showed that the protein utilizes similar interfaces for both nonspecific and specific DNA binding and that the positive patch on the protein surface maintains a similar orientation with the DNA in the two binding modes.¹⁵ In addition, measurements of the one-dimensional diffusion coefficient (D_1) of several DBPs along DNA are consistent with a model that supports rotation-coupled sliding along the helical path of the DNA, which enables the protein to probe the base-pair content in the grooves of the DNA.^{16,17} Molecular dynamic simulations of several proteins with DNA also showed that the electrostatic potential of DBPs is sufficient to orient the protein during

Special Issue: Peter G. Wolynes Festschrift

Received: March 6, 2013

Revised: May 4, 2013

Published: May 13, 2013

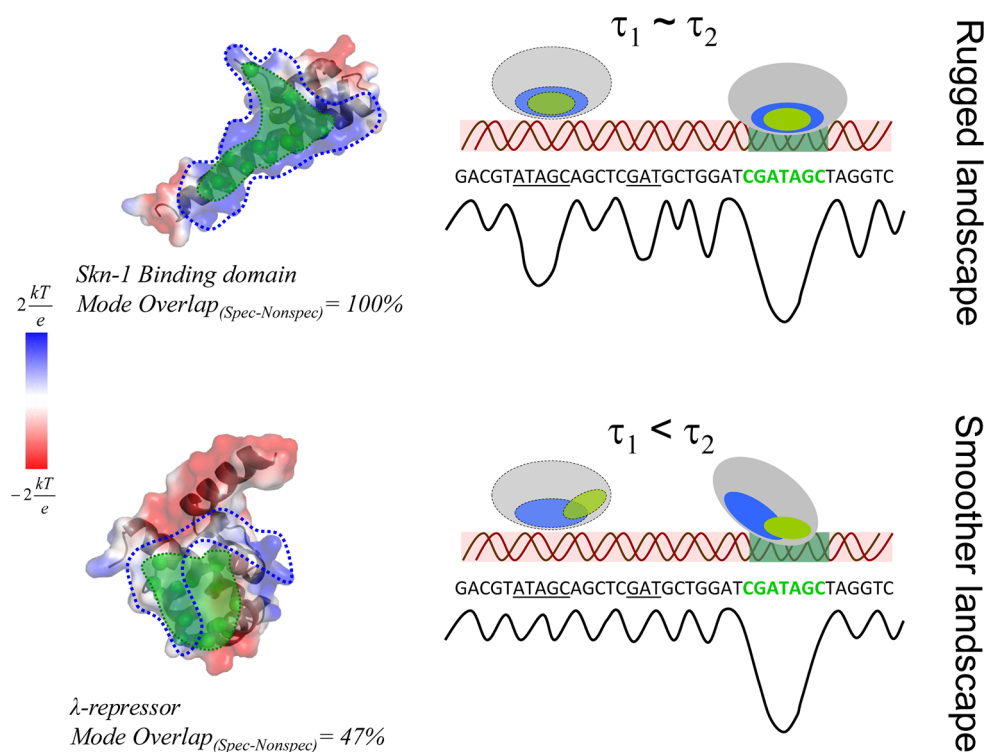


Figure 1. The existence of two protein–DNA binding modes may impose frustration and increase the ruggedness of the protein–DNA energy landscape. Top: Skn-1 TF—a DBP with low frustration between its specific and nonspecific binding modes. Complete overlap (100%; no frustration) is observed between the specific residues (i.e., protein residues forming H-bonds with a specific DNA sequence; green surface) and the nonspecific DNA binding patch (i.e., the positive electrostatic patch that complements the negatively charged DNA backbone, blue surface) on the DBP surface. The scheme on the right illustrates the hypothesized protein–DNA energy landscape with traps corresponding to sites with partial or complete sequence similarity to the cognate binding site of the protein. Bottom: The λ -repressor protein—a DBP with only partial overlap (47%; relatively high frustration) between its specific and nonspecific binding patches. The protein utilizes two distinct patches to slide along DNA and to specifically bind to its cognate sequence. Consequently, the energetic landscape (right) is less rugged and the 1D diffusion is expected to be more rapid. When the frustration is low, the arrival time to the target site (τ_1) and the binding time (τ_2) are similar. When conformational change is needed due to frustration between the specific and nonspecific binding modes, the time to achieve tight binding is longer than the arrival time.

sliding at the binding mode of the specific interaction, and accurately captures the rotation-coupled sliding model.^{18–20}

Despite considerable theoretical^{21–23} and experimental^{24–28} evidence that many DBPs may adopt 1D scanning of DNA interspersed with dissociations into solution, many challenging aspects of the protein–DNA recognition problem remain.²⁹ One example is the contradictory requirement for a DBP to bind with high stability at one specific genomic location and to diffuse rapidly along other locations without falling into the trap of binding at a wrong (albeit similar) site.

Experiments and theory showed that the binding energies of proteins to different DNA sequences span a relatively large range, and can be fitted by a Gaussian distribution with the strongest binding energies in the order of $\sim 30K_bT$ and a standard deviation in the order of $\sim 5K_bT$.^{21,30,31} Such a wide distribution may severely impair the efficiency with which proteins diffuse one-dimensionally by sliding along DNA, as it suggests a highly rugged protein–DNA landscape with deep traps. This conflict, commonly referred to as the “speed–stability” paradox,^{2,21} has been discussed and debated in several works.^{21,31–33} To resolve the conflicting requirements for speed and stability, it has been proposed that DBPs utilize a two-state model for DNA binding separated by a free-energy barrier,³¹ the search state, which corresponds to weak protein–DNA binding and so enables the protein to slide efficiently, and the recognition state, in which the protein is trapped in a high-affinity complex with its cognate regulatory genomic site. The

two distinct binding modes, one for searching and a different one for tight binding, were recently shown by NMR and computational modeling for the Egr-1 zinc-finger transcription factor.³⁴ The two modes differ mostly in the interface the first zinc-finger domain forms with the DNA. While the first finger is much more dynamic than the second and third zinc-finger domains due to the weaker interface when interacting with nonspecific DNA in the search mode, in the recognition mode, its interface is as tight as those fingers 2 and 3 form with the DNA.³⁵

Recently, we investigated the interfaces that DBPs utilize for specific and nonspecific DNA binding (e.g., for sliding).³⁶ Our structural survey of a DBP data set comprising proteins characterized by a variety of folds and functions demonstrated that many proteins have two patches that are involved in DNA interactions: one is characterized by a region of positive potential that is attracted to the negatively charged DNA, and the other is enriched with protein residues that recognize a specific DNA sequence. We found that partial overlap between the two patches is a widespread feature among DBPs, and we demonstrated with molecular-dynamics simulations that increasing the level of frustration between the two binding modes (i.e., reducing the overlap between the binding patches) increases the kinetic barrier to the transition from sliding to specific binding.³⁶

In this work, motivated by earlier theoretical discussions^{21,31,32} and experiments,^{26,37} we assessed the kinetic

effects of partial overlaps between the binding modes on the kinetics of protein sliding along DNA (see Figure 1). We hypothesized that DBPs in which the frustration between the two binding modes is lower (i.e., greater overlap) will experience a more rugged landscape as they slide along DNA, and will more readily bind decoy sites in the genome (sites that share partial or full similarity with the functional cognate site), since they utilize similar modes in both specific and nonspecific binding.³⁶ In contrast, proteins characterized by a higher degree of frustration between the DNA binding modes experience a higher barrier in the transition to specific binding but are less sensitive to sequence variations and so diffuse uninterruptedly along DNA. This work provides important predictive insights into the ability of different proteins to diffuse along DNA, and demonstrates the functional importance of molecular frustration in biomolecular associations.

RESULTS AND DISCUSSION

Specific and Nonspecific DNA Binding Patches Partially Overlap: A Two-State Model for Protein–DNA Interaction. The facilitated diffusion model for rapid searching on DNA posits that the protein alternates between 3D diffusion in solution and 1D excursions of sliding along DNA.² The ability of DBPs to interact with any DNA piece under physiological conditions is enabled through the existence of positively charged patches on their surface that complement the negatively charged DNA backbone. Significant positive surface patches are hallmarks of DBPs and display unique features in comparison with the positive patches that are found in protein–protein interfaces.^{9,10} For example, DBP patches have a higher α -helical content, greater hydrogen-bonding potency, and a higher degree of evolutionary conservation of positively charged residues compared with positive patches in protein–protein interfaces.

Recently, we explored the dual role the positively charged patch may play in contributing to specific contact formation at the target DNA site and in mediating an efficient protein translocation process along the DNA.³⁶ Our goal was to study the similarity between the specific and nonspecific binding modes of proteins to DNA, through an examination of their positive electrostatic patches (which are presumably favored during sliding) and their patches for specific binding (which consist of a group of residues that may form hydrogen bonds and van der Waals interactions with a specific sequence of DNA base pairs).

Here, drawing on the database we created for our earlier study,²⁹ we analyzed a nonredundant data set of 125 DBPs for which the crystal structure with the specific DNA sequence is available.¹⁰ For each DBP, the subgroup of residues that constitutes the patch for specific binding to DNA was defined according to its structure. We evaluated the overlap between the specific and nonspecific protein–DNA binding modes using a measure called χ_{prot} . For each residue i that belongs to the specific mode according to the crystal structure, we calculated χ_i (see Methods), which may range from -1 to 1 and which reflects the enrichment of the spatial environment around residue i with negatively or positively charged amino acids. For each protein, we then calculated χ_{prot} as the mean of the χ_i values for the residues involved in specific DNA binding. Clearly, a protein whose specific and nonspecific DNA patches overlap or even share a mutual common identity (i.e., fully correspond) will have a higher χ_{prot} value. The value of χ_{prot} is also equivalent to the fraction of specific mode residues that

reside in the largest positive patch on the surface of the protein, and reflects the degree of frustration between the specific and nonspecific binding modes of a protein to DNA (i.e., a lower value of χ_{prot} reflects greater frustration).

Figure 2 summarizes the χ_{prot} values of the entire DBP data set (divided into seven different structural/functional groups).

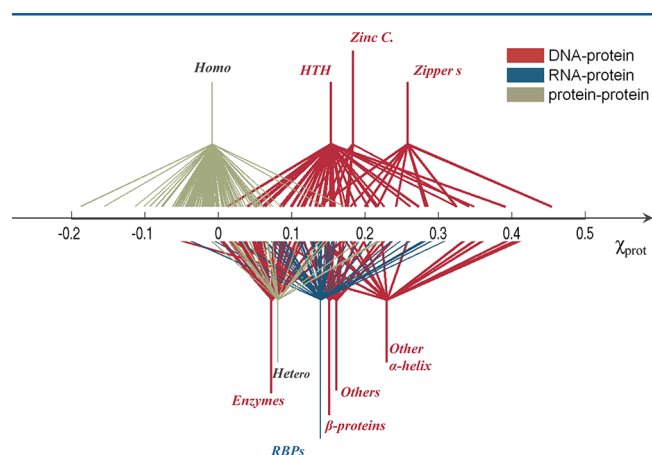


Figure 2. Frustration between specific and nonspecific interfaces in various protein complexes. The analyzed complexes include 125 DBPs (grouped into seven categories based on their fold and function), 37 RNA-binding proteins (RBPs), and 145 protein–protein complexes (129 homodimers, Homo, and 16 heterodimers, Hetero). The conflict between the specific and nonspecific binding patches is evaluated by the values of χ_{prot} with a higher χ_{prot} indicating lower frustration between the two DNA binding modes and a higher percentage overlap between the two patches (see Figure 1). While the distribution of the DBPs is shifted to higher and more positive values, many DBPs present little overlap between their specific and nonspecific DNA binding modes, which may create a kinetic barrier to the transition from sliding to specific DNA-target binding.³⁶

For reference purposes, we also carried out the analysis on three additional data sets: 37 RNA-binding proteins (RBPs),³⁸ 16 heterodimers in which the macromolecular protein–protein interface is dominated by electrostatic interactions,³⁹ and 129 homodimeric protein complexes in which the interfacial electrostatic patches discussed above are expected to be negligible⁴⁰ (see Methods for more details on the protein data sets).

Figure 2 shows higher χ_{prot} values for DBPs than for RBPs and protein–protein complexes, as protein interfaces for DNA binding are, on average, more enriched with positive charges than are interfaces for RNA and protein binding. However, DBPs exhibit mostly moderate χ_{prot} values (ranging from approximately 0.05 to 0.25). We find that, in many DBPs, the observed χ_{prot} is less than the maximal χ_{prot} available from an optimal rearrangement of the charges along the sequence. This indicates the existence of partial frustration between specific and nonspecific DNA binding, as many neutral and negatively charged residues may surround the positively charged residues that specifically bind DNA. Although examples exist for negatively charged residues in protein–DNA interfaces,⁴¹ these surrounding residues may introduce local electrostatic repulsion from the DNA. We provided several examples that showed, through molecular dynamic simulations, that proteins with lower χ_{prot} values may adopt distinct conformations in specific and nonspecific DNA binding.³⁶ Thus, the observation of less than maximal patch overlap suggests an implicit conflict within DBPs between their specific and nonspecific DNA-

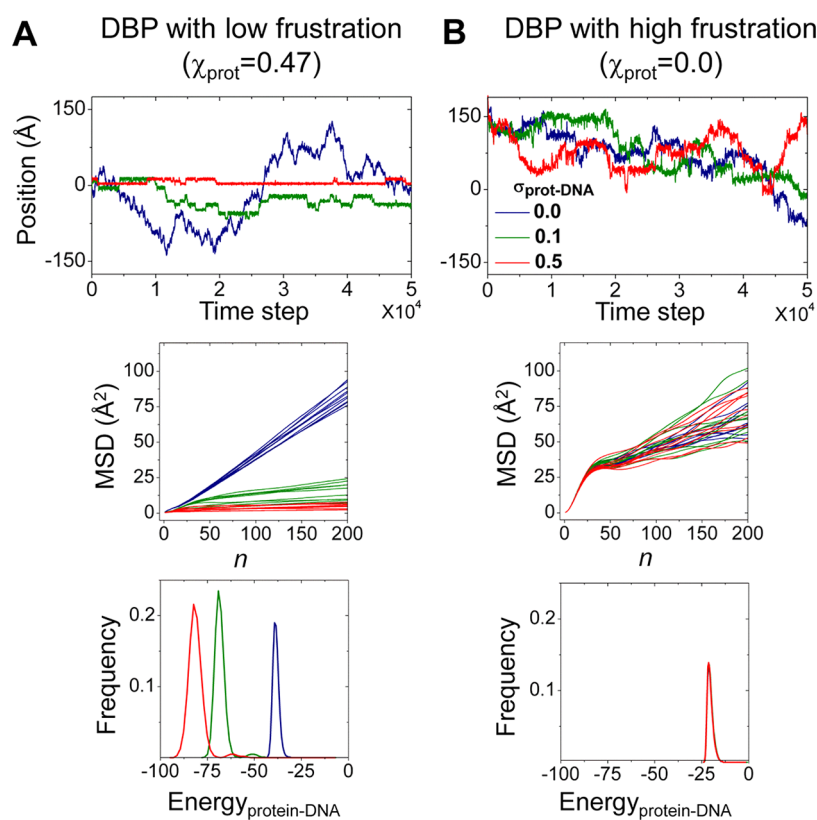


Figure 3. Proteins with lower frustration between their nonspecific and specific binding modes are more sensitive to the details of the energy landscape. (A) Top: Trajectories of Skn-1 with $\chi_{\text{prot}} = 0.47$ on a nonspecific DNA strand (blue) and on DNA strands with a landscape ruggedness of $\sigma_{\text{prot-DNA}} = 0.1$ (green) and $\sigma_{\text{prot-DNA}} = 0.5$ (red). The increased ruggedness of the landscape creates traps that impede protein translocation along the axis of the DNA. Middle: Mean square displacement profiles (MSD) for 10 representative trajectories of the three strengths of ruggedness. The decreased slope reflects the fact that the 1D diffusion coefficient for protein sliding along DNA decreases considerably with increasing $\sigma_{\text{prot-DNA}}$. Bottom: Histograms of total protein–DNA energies (electrostatics and van der Waals energies) for the three conditions. The average energy decreases with increasing $\sigma_{\text{prot-DNA}}$ as the protein probes the base content during nonspecific binding. (B) Same as in part A but for uracil-DNA glycosylase, with $\chi_{\text{prot}} = 0$. The 1D diffusion of the protein along DNA is unaffected by the ruggedness of the landscape ($\sigma_{\text{prot-DNA}}$). The mean protein–DNA energy is unchanged as the specific DNA patch on the surface of the protein is segregated from the patch the protein utilizes for sliding.

binding modes, which may raise a kinetic barrier to the protein switching from sliding along DNA to specific binding of a DNA sequence.

Proteins with Moderate Frustration Efficiently Diffuse along DNA. In an alphabet of four letters, a 10-bp-long target DNA sequence will occur once per $\sim 10^6$ nucleotides. Thus, promiscuous binding sites sharing full or partial sequence identity with a cognate target for a DBP will occur on the DNA and may hinder the search process by trapping the protein during its 1D movement along DNA.⁴² Previous theoretical studies suggest that a two-state model for protein–DNA interactions (i.e., two distinct protein–DNA conformations, one for sliding and another for specific binding) is necessary to resolve the speed–stability paradox^{21,32,35,43} and to overcome the binding of decoy genomic sites. Alternatively, strong coupling between 3D and 1D modes of search leading to frequent binding and unbinding events of the protein from the DNA was recently shown to maintain rapid exploration of many DNA sites.³³

Here, we studied the relationship between the specific–nonspecific DNA binding frustration of the protein described above and the quality of its 1D movement along DNA with varying sequences. Specifically, we anticipated that proteins whose specific and nonspecific patches do not overlap to any

great extent (low χ_{prot} , high frustration) and that would therefore tend to utilize different conformations during sliding³⁶ versus binding to the cognate site would be less interrupted by energetic noise caused by variation in the sequence as they slide along DNA. In contrast, we expected that proteins with a high degree of overlap between their specific and nonspecific DNA binding patches (high χ_{prot} , low frustration), and whose specific DNA binding patch therefore constantly faces the DNA molecule while sliding, would be more easily trapped in semispecific sites.

To test our hypothesis, we performed coarse-grained molecular dynamics sliding simulations to assess the 1D diffusion along DNA of proteins from the database: three with low frustration (Skn-1, Pax, and Sap-1 with $\chi_{\text{prot}} > 0.2$) and another three with higher frustration (Spo0A, uracil DNA glycosylase (UDG), and λ -repressor with $\chi_{\text{prot}} \sim 0$). In the simulation model, we supplemented the electrostatic interactions acting between the charged residues and any of the DNA phosphates with an attractive potential that represented sequence readout interactions (e.g., hydrogen bonds, van der Waals, and hydrophobic forces) between each of the DNA bases and each of the protein residues belonging to the specific DNA binding patch. Interactions between the protein residues and DNA bases were modeled by an attractive Lennard-Jones

term, where each individual pair interaction was assigned a potential strength (ϵ_{ij}) drawn from a Gaussian distribution. We modified the ruggedness of the protein–DNA energy landscape by changing the standard deviation of the distribution (σ) and measured how the properties of the one-dimensional diffusion along the DNA were affected (for additional details, see Methods).

Figure 3 demonstrates the propagation of two proteins (Skn-1, $\chi_{\text{prot}} = 0.47$; UDG, $\chi_{\text{prot}} = 0$) along DNA. The simulations include electrostatic interactions between the charged residues and phosphate beads alone and in combination with some or many specific protein interactions with DNA bases (specific DNA interactions, modeled by increasing values of σ). In the presence of only nonspecific DNA interactions, the two proteins cover relatively large segments of the DNA (Figure 3A and B, blue line). As specific interactions are randomly introduced between the specific patch residues of Skn-1 and DNA bases (drawn from a Gaussian distribution with $\langle \epsilon_{ij} \rangle = 0.5$ and $\sigma = 0.1$), the protein is biased to specific locations along the DNA and covers a much lower fraction of DNA by 1D scanning (Figure 3A, green line). As the distribution of the energetics of specific interactions further broadens ($\sigma = 0.5$), Skn-1 ceases to propagate one-dimensionally along DNA because it is trapped in a single location by the rugged energy landscape (Figure 3A, red line). In contrast to Skn-1, which has relatively low frustration between its two DNA binding modes, UDG is much more frustrated, with little overlap between its specific and nonspecific patches for DNA binding. As it moves one-dimensionally along DNA, UDG interacts with the DNA through an electrostatic patch segregated from its specific DNA-recognition patch. Consequently, UDG is much less sensitive to sequence variations (i.e., to specific interactions with DNA bases, which increase with increasing σ) in comparison with Skn-1, and the quality of its DNA scanning is unaffected (Figure 3B). The effects of landscape noise (σ) on the 1D diffusion coefficient can be inferred from Figure 3 (middle), which show the mean square displacement (MSD) profiles of the two proteins along the DNA axis. While the slopes of the profiles for Skn-1 are significantly lower in a noisy environment ($\sigma = 0.1, 0.5$) than the profiles with nonspecific DNA, they remain similar in all three environments for UDG. Moreover, the protein–DNA energies sampled throughout the 1D scan along DNA (the sum of the specific and nonspecific electrostatic energies) decrease for Skn-1 as the noise increases but remain unchanged for UDG because its “sticky” patch for specific DNA recognition is entirely uninvolved in the nonspecific DNA interactions that predominate during sliding (Figure 3, bottom).

The observations for Skn-1 and UDG imply that proteins with low frustration experience a rugged landscape for sliding along DNA that reduces their 1D diffusion coefficient.⁴⁴ Figure 4 demonstrates, using proteins with varying degrees of frustration, how the ruggedness (σ) of the free energy landscape in which the sliding takes place may reduce 1D diffusion by trapping the protein at randomly positioned sites on the DNA that resemble its cognate binding site. For each sliding event of a protein along DNA (defined as a 1D excursion that may involve sliding and short-range hopping), we use a measure, d_z ,¹⁹ that is the distance between the minimal and maximal positions on the DNA axis scanned by the protein during the sliding event (see Methods). A protein whose 1D diffusion along DNA is hindered by traps at some sites on the DNA because of increasing ruggedness or noise ($\sigma > 0$) is

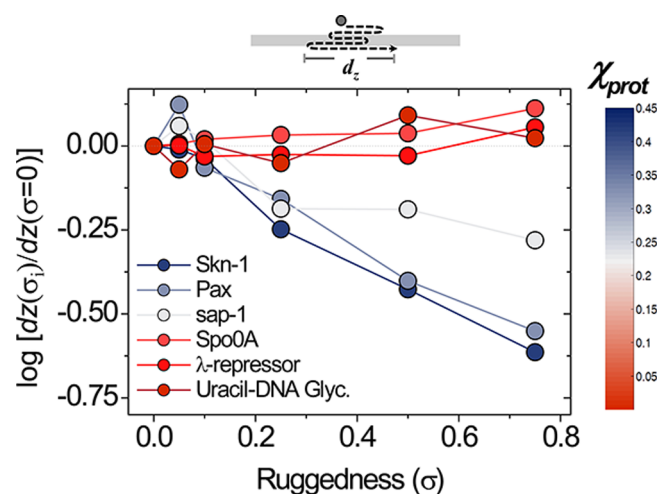


Figure 4. Trapping of proteins with varying degrees of frustration (χ_{prot} ; defined on a color scale at the right of the graph) during sliding along DNA. The propensity for a protein to be trapped at certain positions on DNA is evaluated as the log ratio of the average $d_z(\sigma_i)$ under a given condition to the average $d_z(\sigma=0)$ calculated for protein sliding along a DNA segment with minimal ruggedness. Proteins with a large overlap between their specific and nonspecific binding patches (higher χ_{prot} ; less frustration) utilize similar interfaces for both sliding and specific DNA binding. Consequently, they tend to get trapped as they move one-dimensionally along DNA more often and for longer than do proteins with less overlap (lower χ_{prot} ; greater frustration). The studied proteins are Skn-1 (1SKN), Pax (1FJL), sap-1 (1BC8), Spo0A (1LQ1), λ -repressor (1LMB), and uracil-DNA glycosylase (1EMH).

expected to have a lower d_z value compared with that measured when noise is minimal ($\sigma = 0$). By contrast, proteins that are able to maintain similar 1D random walk behavior in all energy landscapes are expected to exhibit the same d_z value in both more and less rugged environments. Figure 4 shows the ratio between the averages of d_z for a rugged DNA ($\langle d_z \rangle_\sigma$) and for a smooth DNA ($\langle d_z \rangle_{\sigma=0}$). For proteins with substantial overlap between their specific and nonspecific DNA binding patches (low frustration, high χ_{prot}), such as Skn-1, SAP-1, and a subunit of the homeodomain of the *Drosophila* paired protein (pax), d_z significantly decreases as the noise in the energetic landscape of the specific protein–DNA interaction increases. The 1D diffusion along DNA of proteins with high frustration between their specific and nonspecific binding modes (low χ_{prot}), such as the effector domain of Spo0A, UDG, and a subunit from the λ -repressor, is less affected by noise in the energy landscape.

Weak Frustration Imposes a Kinetic Barrier in the Transition from Nonspecific to Specific DNA Binding.

Partial overlap between a surface patch that is favored during protein sliding on DNA (i.e., nonspecific binding) and another that is mostly activated upon specific sequence binding (Figure 2) might imply the existence of conformational differences between the two DNA binding modes.^{5,6,36,45} In that case, recognition of a cognate DNA sequence by a protein undertaking 1D sliding might become nontrivial^{146,47} and might result in a delay between target localization and target binding, which would further complicate the kinetic challenge in protein–DNA recognition.²

Using coarse-grained molecular-dynamics simulations, we evaluated the effects of the overlap between specific and nonspecific DNA binding modes on the kinetics of transition between the two modes.³⁶ We designed an *in silico* library of

150 variants of the recognition helix of MAD (composed of 26 residues) from the heterodimer MAD-MAX, in which we modified the amount and positioning of positively and negatively charged residues along the sequence while maintaining the protein–DNA specific contact map without modification (Figure 5). Each peptide in the library has

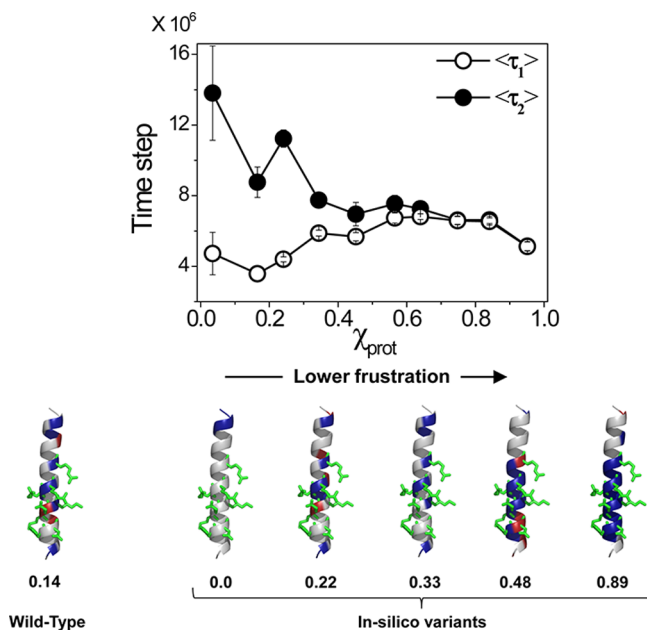


Figure 5. The relationship between the degree of frustration and search kinetics. The search kinetics is quantified by the first passage times for target localization (τ_1) and target binding (τ_2) calculated from simulations of *in silico* designed peptides. The larger time gap ($\tau_2 - \tau_1$) for peptides with higher frustration (i.e., lower χ_{prot}) indicates the existence of a transition time during which the protein switches from nonspecific to specific binding. The search kinetics was studied for various variants of recognition helix of the bHLHZip heterodimer MAD-MAX. In all of these cases, ruggedness of the energy landscape of the DNA is zero (i.e., $\sigma = 0$). The degree of frustration between the residues that participate in specific and nonspecific binding is illustrated for some peptide variants. The specific DNA binding patch is unaltered, while the nonspecific patch is modified. Residues forming specific binding contact with DNA are represented in green by a stick representation. Negatively charged residues are shown in red, while positively charged residues are shown in blue.

effectively a different degree of overlap between its specific mode residues and its charged residues, and the library is designed such that the peptides span χ_{prot} values in the range 0–1. We then studied the binding kinetics in the peptides by individually simulating each protein with a 100 bp dsDNA that contains a binding site for the helix in the middle. The times for two events were then measured for each of the peptides. The first was the time elapsed from the start of the search until the protein localized the target site location (τ_1), and the second was the time elapsed from the start of the search until specific protein binding was achieved (τ_2) (defined as the formation of >75% of the specific protein–DNA contacts in the middle of the DNA).

The *in silico* model of the wild-type peptide (both in terms of the number of charges and their positioning on the 26-residue helix) displays a moderate degree of frustration between the two modes ($\chi_{\text{prot}} \sim 0.1$) and, on average, a marked time gap between target site localization and binding (i.e., $\tau_2 > \tau_1$).³⁶ Owing to the limited overlap between the positively charged residues that interact with the DNA as the protein slides along it and the residues forming specific DNA contacts, the orientation of the helix during sliding is not fully compatible with the orientation it adopts during specific binding. Consequently, the protein is observed to overshoot its target several times, or bind it weakly (protein–contact formation $\ll 75\%$) and dissociate for additional sliding periods. Nevertheless, specific binding is finally achieved as the helix may stochastically sample the appropriate orientation for specific binding, in accordance with previous theory.³¹

As the overlap between the modes becomes more complete (i.e., as χ_{prot} increases), the time elapsed from locating the target to fully binding it reduces to zero ($\tau_2 \sim \tau_1$) and the kinetics of binding becomes a direct, first-passage problem (Figure 5). A greater amount of overlap between the positively charged residues and the specific mode residues (i.e., lower frustration) reduces competition between the two patches for DNA binding during protein sliding versus specific binding and makes the specific and nonspecific binding mode conformations more compatible with one another.³⁶ Presumably, greater overlap between the two modes also facilitates the stochastic transition between them. Consequently, the protein performs sliding while its specific mode residues are in closer contact with the

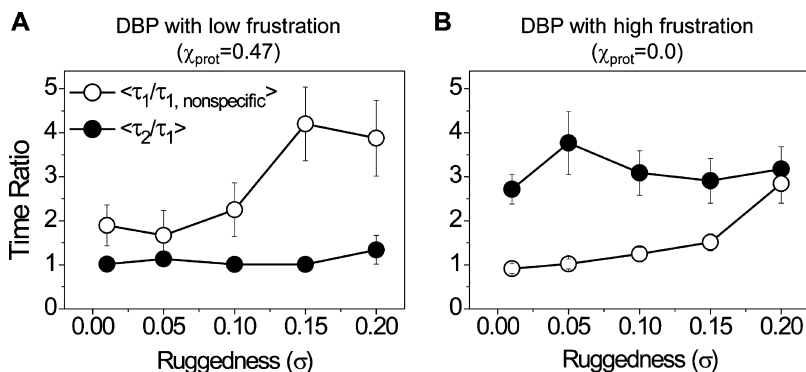


Figure 6. Interplay between frustration and ruggedness and their effect on search kinetics. The kinetics of the search is studied for two proteins: Skn-1, with $\chi_{\text{prot}} = 0.47$ (A) and uracil-DNA glycosidase, with $\chi_{\text{prot}} = 0$ (B). The search kinetics is quantified by two time ratios: $\tau_1(\sigma)/\tau_1(\sigma=0)$ and $\tau_2(\sigma)/\tau_1(\sigma)$. The former indicates the time to arrive at the target site when searching rugged DNA (the degree of ruggedness is dictated by σ) normalized by the arrival time for smooth DNA. The latter is the ratio between the time needed to form a specific complex and the time needed to arrive for each value of σ .

DNA and may directly transfer from sliding to specific binding at its first encounter with a cognate DNA sequence.

Weak Frustration Might Resolve the Speed-Stability Paradox. The degree of frustration in DBPs affects both the 1D diffusion on DNA (Figures 3 and 4) and the rate of achieving specific binding (Figure 5). While the degree of ruggedness of the DNA is expected to severely affect the sliding dynamics, it is expected to have only a minor effect on the rate of forming the specific binding at the target site. We therefore studied the times for arriving and recognizing the target site (τ_1 and τ_2 , respectively), for two proteins with low and high degrees of frustration when searching DNA sequences of different degrees of ruggedness. For a simple comparison between the two proteins, we normalized τ_1 by τ_1 when sliding on a smooth DNA molecule (i.e., $\sigma = 0$) and τ_2 by τ_1 obtained for the same value of σ . If $\tau_1(\sigma)/\tau_1(\sigma=0)$ equals 1, the sliding of the DBP is not affected at all by the ruggedness. As this ratio increases, the DBP is more trapped in local minima. If $\tau_2(\sigma)/\tau_1(\sigma)$ equals 1, the DBP recognizes the binding site immediately when it arrives to the site. A ratio larger than 1 indicates the incorporation of a conformational transition.

Figure 6 shows that, for a DBP with low frustration, the search time to find the target site, τ_1 , increases together with the increase in ruggedness. For a DBP with high frustration, the ruggedness has a minor effect as the protein is less sensitive to local minima in the protein–DNA energy landscape. An opposite effect is observed for the time needed to achieve specific binding at the target site. When frustration is low, recognition occurs immediately after the DBP arrives at the binding site, for any degree of ruggedness. However, when frustration is high, recognition is delayed due to the need to switch from the nonspecific binding mode to the specific binding mode.

CONCLUSIONS AND IMPLICATIONS

The mechanism of facilitated diffusion provides a physical framework for the rapid search of a protein for its target site on the DNA molecule that includes many potential binding sites. The intrinsic complexity of the search performed by DBPs can be even further increased by various *in vivo* factors.^{48,49} In this article, we discuss two additional aspects for the DNA search mechanism. First, we argue that the process of target site localization is not always reduced to simply finding the correct site because it may require in addition formation of the unique interface between the DBP and the DNA. Accordingly, we suggest that the arrival time at the target site and the recognition time are not necessarily identical. The implication of the decoupling between the arrival and recognition time scales is that not all localization of the target site may end up with productive binding. Recent observation of the *lac*-repressor in living cells shows that the protein slides several times over its functional operator *LacO1* before binding.⁴⁶ Second, the search time is not only affected by the number of sites that have to be scanned (i.e., the length of the DNA and its structure) but also by the ruggedness of the protein–DNA landscape for the search. Some sites may act as traps that slow down the search.

The lag time between localizing the target site and the time it takes to form the specific complex together with the idea that search might take place on a rugged landscape may both contribute to a reduced search speed. In this study, we show that these two aspects of DNA search complexity are related to each other and can be modulated by the molecular properties

of the proteins that perform the search. Several experiments suggest that nonspecific and specific protein–DNA binding modes may share similar structural properties,¹⁵ while others highlight discrepancies between the two modes in either the orientation of the protein to the DNA^{5,45,50} or the conformations the molecules adopt.^{6,51} We show that the existence of two modes of interaction with the DNA controls the sliding on rugged DNA and lag time between arriving and binding the site.

Our structural survey indicates that DBPs display a wide range of overlaps between their positive electrostatic patch and the patch involved in specific DNA sequence readout.³⁶ We argue that, as the overlap between the specific and nonspecific DNA binding modes of a DBP decreases (high frustration; a lower value of χ_{prot}), the ease with which the protein can navigate through the noisy DNA landscape increases. Consequently, the protein is able to reach its DNA target relatively quickly without becoming trapped at decoy sites. We showed that while the frustration smoothens the landscape for sliding it induces a greater conformational difference between the sliding mode and specific complex with a cognate DNA sequence. Under such conditions, the transition from 1D DNA scanning to specific binding at a target site may require that the protein undergo a conformational switch or change in orientation relative to the DNA via crossing a free energy barrier. However, proteins with low frustration will switch from sliding to specific binding faster, but they may be less selective toward binding at semispecific sites and their 1D diffusion is significantly slower or nonexistent in extremely rugged energy landscapes.

Evidence for the existence of two modes of interaction with DNA can be found in several structural and spectroscopic studies,^{5,6,34} computer simulations,^{45,52} as well as several theoretical studies that indicate the necessity of a two-state protein–DNA binding model for efficient 1D motion by the protein along DNA.^{9,31,32} We quantified the effect of the overlap between the two-binding modes (i.e., specific and nonspecific patches) on search kinetics through simulations involving a library of *in silico* designed variants of a DNA recognition helix³⁶ and a set of natural DBPs with different values of χ_{prot} . We showed that greater frustration between the two DNA binding modes results in (1) a smoother landscape for sliding and consequently faster 1D diffusion and (2) a time lag emerging between target localization and target binding. In contrast, low frustration between the specific and nonspecific binding modes results in a sluggish sliding and a rapid transition from sliding to specific binding in what can be envisioned as a “lock-and-key” mechanism. An example for such multistate kinetics of DNA sequence recognition has been described earlier for the C-terminal domain of the papillomavirus E2 protein with its target DNA sequence.⁴⁷ We propose here that one consequence of low overlap between the two DNA binding modes is some degree of “plasticity” at the protein–DNA interface, in what could be considered an “induced fit” mechanism. Such “plasticity” comes into play in the form of either DNA bending and/or protein conformational changes that are common in many specific protein–DNA complexes, and which aim to maximize the formation of stabilizing interactions at the interface and so facilitate the switch to specific binding.

Our study serves as another example of the important role of frustration in biomolecular systems.^{53–55} Wolynes and his co-workers have shown for various protein systems that on the one

hand frustration should be minimized (e.g., to allow an efficient folding) but on the other hand nonzero frustration is essential for function (e.g., allostery and conformational transition).^{56–61} The analysis of the rates of sliding and of binding specifically on smooth or rugged DNA molecules shows the importance of frustration of DBPs in preventing slow kinetics. DBPs with low frustration may experience a very sluggish dynamics due to trapping in local minima. DBPs with high frustration may experience a slow transition rate. In line with previous theory,^{21,31,32} we propose that a moderate degree of frustration may compromise between these two factors and result in a fast binding rate. Nonetheless, one may hypothesize that DBPs may exhibit different degrees of frustration that may affect the speed of the search in different ways to support the function of each protein. For example, DBPs with a higher degree of frustration might be linked with cases in which fast DNA is not essential and “wasteful” commitments to promiscuous genomic sites is not a severe limitation. In these cases, finding the target site will follow very fast binding. We suggest that the degree of frustration in DBPs has to be quantified and analyzed to better understand their biophysics and function in the cell.

METHODS

Protein Data Sets. We utilized a nonredundant data set¹⁰ of 125 DBPs (<35% pairwise sequence identity) cocrystallized with their specific DNA sequence at a resolution of <3 Å to study the frustration between specific and nonspecific DNA binding.³⁶ The electrostatic properties observed in DBPs were compared with those of RNA binding proteins and protein dimers. We used a nonredundant data set of 37 RNA-binding proteins (RBPs).³⁸ Protein–protein complexes were represented by homo- and heterodimeric proteins. A data set of 129 homodimeric protein chains⁴⁰ was analyzed as a reference data set, since homodimers are not expected to have significant patches of positively charged electrostatic potential. To complement the analysis of protein–protein complexes, we analyzed 16 heterodimeric proteins with high electrostatic free energies for binding (less than -5 kcal/mol).³⁹ A cutoff criterion of a 4 Å intermolecular distance between the heavy atoms of a protein residue and any heavy atom from its neighboring interacting macromolecule (i.e., a residue or base from another protein chain, DNA, or RNA) was used to define the interfaces.

Calculation of Specific and Nonspecific Patch Overlap and Frustration (χ_{prot}). For each protein in the data set, we calculated χ_i according to

$$\chi_i = \frac{\sum_{j'} q_{j'} \exp\left(-a \frac{r_{ij'}}{r_c}\right)}{\sum_{j'} \exp\left(-a \frac{r_{ij'}}{r_c}\right)}$$

where j' denotes all the residues whose C_α atom is closer than a cutoff distance of $r_c = 10$ Å to the C_α atom of residue i . The value $q_{j'}$ is a point charge of 1, -1 , or 0, $r_{ij'}$ is the C_α – C_α distance between residues i and j' , and $a = 2$ is an exponential decay constant. A χ_i value approaching 1 is therefore expected for residues that are fully surrounded by positively charged residues. The value of χ_{prot} is the mean of the χ_i values for protein residues that belong to the interface used for specific binding to the DNA.³⁶

To complement the χ_{prot} measure, we also calculated the overlap between the patch for specific binding and the

positively charged patch for each DBP in the data set. We used the PatchFinder⁹ package and Poisson–Boltzmann calculations to identify the largest positive patch in each protein. The overlap between the specific and nonspecific DNA binding patch was then calculated as the fraction of residues that belong to both the specific patch (according to the crystal structure of the specific protein–DNA complex) and the largest positive patch.

Coarse-Grained Molecular Dynamic Simulation Model. In this work, we sought to investigate the relatively long biological time scales of protein–DNA recognition dynamics, a task which is still computationally challenging for most atomistic simulation packages.⁶² We therefore employed a coarse grained approach and simulated the dynamics of the system with the Langevin equation. We modeled dsDNA (in most cases 100 bp long) with three beads per nucleotide, representing the sugar, phosphate, and base. Each bead was positioned at the geometric center of the group it represented. The DNA was held static throughout the simulations and was aligned with and centered on the Z-axis. The protein was represented by a single bead for each residue located at the C_α of that residue, and was simulated by a native topology-based model that excludes nonnative interactions.^{18,19} This model, which eliminates degrees of freedom for the system and compromises on the level of detail, has been successfully employed in previous works to structurally characterize protein sliding along DNA,^{18,19} the enhancement of Arc-repressor dimerization by DNA,⁶³ and tail mediated intersegmental transfers by proteins between adjacent DNA molecules.⁶⁴

We represented native interactions (in the protein and between the protein and DNA at the specific binding site only) in our model by the Lennard-Jones potential (with $\epsilon = 1$) and non-native interactions by a hard-sphere repulsion term (C/r_{ij})¹², where $C = 4$ Å for a C_α to C_α collision and $C = 5.7$ Å for a C_α to DNABead collision.^{18,19} Our model is supplemented with intraprotein and protein–DNA nonspecific electrostatic interactions. The positively charged residues of the protein (Lys, Arg) were assigned a point charge of $+1$, and the negatively charged residues (Asp, Glu) as well as the phosphate beads of the DNA backbone were assigned a negative charge of -1 . The electrostatic potential between charged beads q_i and q_j was modeled by the Debye–Hückel interaction, which accounts for the ionic strength of a solute immersed in aqueous solution. A relatively low ionic strength of $C_s = 0.01$ M was used in our simulations, to focus on one-dimensional movements of protein on DNA.

Simulations with DNA Sequence Disorder. To account for variations in DNA sequence and how this may affect the landscape for 1D movement by proteins with distinct properties, we simulated several proteins characterized by varying degrees of frustration between their binding patches on a 100 bp dsDNA. We defined native-like interactions between each of the protein residues belonging to the specific DNA binding patch and each DNA bead representing a DNA base. For each such interaction pair within the total $n_{\text{sp}} \cdot b$ interactions (where n_{sp} is the number of residues forming specific contacts with DNA according to the crystal structure and b is the number of beads representing DNA bases), we assigned a value for ϵ_{ij} in the Lennard-Jones potential term. We drew the ϵ_{ij} values from a Gaussian distribution ($F(\epsilon) = (1/(2\pi\sigma^2)^{1/2}) \exp[-((\epsilon - \epsilon_{\text{ns}})^2/2\sigma^2)]$) with mean $\langle \epsilon_{ij} \rangle = 0.5$ and a standard deviation ($\sigma \equiv \sigma_{\text{prot-DNA}}$) of 0, 0.05, 0.1, 0.25, 0.5, or 0.75.

Analysis of 1D Excursions of Protein along DNA. A rugged energy landscape will reduce the 1D diffusion coefficient for protein movement along DNA. Since, in the simulations of the protein on extremely rugged landscapes, we observed 1D diffusions of the protein along DNA that deviated from random behavior, we employed an alternative measure (d_z) to calculate how ruggedness affected sliding¹⁹ rather than directly calculating the diffusion coefficients from the mean square displacement profiles.¹⁸ The alternative measure, d_z , is the distance between the maximal and minimal positions on the Z-axis visited by the protein during the sliding event (see the illustration in Figure 5). A protein that is trapped in a minimum during DNA sampling will have a lower average d_z/τ_{1D} (where τ_{1D} is the duration of a single 1D excursion). In contrast, proteins maintaining their random walk behavior despite an increase in the ruggedness of the landscape will show no decrease in their average d_z/τ_{1D} value.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Koby.Levy@weizmann.ac.il

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Kimmelman Center for Macromolecular Assemblies and the Minerva Foundation with funding from the Federal German Ministry for Education and Research. Y.L. is the incumbent of the Lillian and George Lyttle Career Development Chair.

REFERENCES

- (1) Riggs, A. D.; Bourgeois, S.; Cohn, M. Lac Repressor-Operator Interaction. 3. Kinetic Studies. *J. Mol. Biol.* **1970**, *53* (3), 401–48.
- (2) Berg, O. G.; Winter, R. B.; Vonhippel, P. H. Diffusion-Driven Mechanisms of Protein Translocation on Nucleic-Acids. 1. Models and Theory. *Biochemistry* **1981**, *20* (24), 6929–6948.
- (3) Vonhippel, P. H.; Berg, O. G. Facilitated Target Location in Biological-Systems. *J. Biol. Chem.* **1989**, *264* (2), 675–678.
- (4) Winkler, F. K.; Banner, D. W.; Oefner, C.; Tsernoglou, D.; Brown, R. S.; Heathman, S. P.; Bryan, R. K.; Martin, P. D.; Petratos, K.; Wilson, K. S. The Crystal-Structure of Ecorv Endonuclease and of Its Complexes with Cognate and Non-Cognate DNA Fragments. *EMBO J.* **1993**, *12* (5), 1781–1795.
- (5) Viadiu, H.; Agarwal, A. K. Structure of BamHI Bound to Nonspecific DNA: A Model for DNA Sliding. *Mol. Cell* **2000**, *5* (5), 889–895.
- (6) Kalodimos, C. G.; Biris, N.; Bonvin, A. M. J. J.; Levandoski, M. M.; Guennegues, M.; Boelens, R.; Kaptein, R. Structure and Flexibility Adaptation in Nonspecific and Specific Protein-DNA Complexes. *Science* **2004**, *305* (5682), 386–389.
- (7) Record, M. T.; Ha, J. H.; Fisher, M. A. Analysis of Equilibrium and Kinetic Measurements to Determine Thermodynamic Origins of Stability and Specificity and Mechanism of Formation of Site-Specific Complexes between Proteins and Helical DNA. *Methods in Enzymology* **1991**, *208*, 291–343.
- (8) Misra, V. K.; Hecht, J. L.; Yang, A. S.; Honig, B. Electrostatic Contributions to the Binding Free Energy of the Lambda cI Repressor to DNA. *Biophys. J.* **1998**, *75* (5), 2262–2273.
- (9) Stawiski, E. W.; Gregoret, L. M.; Mandel-Gutfreund, Y. Annotating Nucleic Acid-Binding Function Based on Protein Structure. *J. Mol. Biol.* **2003**, *326* (4), 1065–1079.
- (10) Szilagy, A.; Skolnick, J. Efficient Prediction of Nucleic Acid Binding Function from Low-Resolution Protein Structures. *J. Mol. Biol.* **2006**, *358* (3), 922–933.
- (11) Jones, S.; van Heyningen, P.; Berman, H. M.; Thornton, J. M. Protein-DNA Interactions: A Structural Analysis. *J. Mol. Biol.* **1999**, *287* (5), 877–896.
- (12) Garvie, C. W.; Wolberger, C. Recognition of Specific DNA Sequences. *Mol. Cell* **2001**, *8* (5), 937–946.
- (13) Lundback, T.; Hard, T. Sequence-Specific DNA-Binding Dominated by Dehydration. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93* (10), 4754–4759.
- (14) Luscombe, N. M.; Laskowski, R. A.; Thornton, J. M. Amino Acid-Base Interactions: a Three-Dimensional Analysis of Protein-DNA Interactions at an Atomic Level. *Nucleic Acids Res.* **2001**, *29* (13), 2860–2874.
- (15) Iwahara, J.; Zweckstetter, M.; Clore, G. M. NMR Structural and Kinetic Characterization of a Homeodomain Diffusing and Hopping on Nonspecific DNA. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (41), 15062–15067.
- (16) Blainey, P. C.; Luo, G. B.; Kou, S. C.; Mangel, W. F.; Verdine, G. L.; Bagchi, B.; Xie, X. S. Nonspecifically Bound Proteins Spin while Diffusing along DNA. *Nat. Struct. Mol. Biol.* **2009**, *16* (12), 1224–U34.
- (17) Bagchi, B.; Blainey, P. C.; Xie, X. S. Diffusion Constant of a Nonspecifically Bound Protein Undergoing Curvilinear Motion along DNA. *J. Phys. Chem. B* **2008**, *112* (19), 6282–6284.
- (18) Givaty, O.; Levy, Y. Protein Sliding along DNA: Dynamics and Structural Characterization. *J. Mol. Biol.* **2009**, *385* (4), 1087–1097.
- (19) Marcovitz, A.; Levy, Y. Sliding Dynamics along DNA: A Molecular Perspective. In *Innovations of Biomolecular Modeling and Simulations*; RSC: 2012; Vol. 2.
- (20) Terakawa, T.; Kenzaki, H.; Takada, S. p53 Searches on DNA by Rotation-Uncoupled Sliding at C-Terminal Tails and Restricted Hopping of Core Domains. *J. Am. Chem. Soc.* **2012**, *134* (35), 14555–14562.
- (21) Slutsky, M.; Mirny, L. A. Kinetics of Protein-DNA Interaction: Facilitated Target Location in Sequence-Dependent Potential. *Biophys. J.* **2004**, *87* (6), 4021–4035.
- (22) Sheinman, M.; Benichou, O.; Kafri, Y.; Voituriez, R. Classes of Fast and Specific Search Mechanisms for Proteins on DNA. *Rep. Prog. Phys.* **2012**, *75*, 2.
- (23) Kolomeisky, A. Physics of Protein-DNA Interactions: Mechanisms of Facilitated Target Search. *Phys. Chem. Chem. Phys.* **2011**, *13*, 2088–2095.
- (24) Elf, J.; Li, G. W.; Xie, X. S. Probing Transcription Factor Dynamics at the Single-Molecule Level in a Living Cell. *Science* **2007**, *316* (5828), 1191–1194.
- (25) Tafvizi, A.; Huang, F.; Leith, J. S.; Fersht, A. R.; Mirny, L. A.; van Oijen, A. M. Tumor Suppressor p53 Slides on DNA with Low Friction and High Stability. *Biophys. J.* **2008**, *95* (1), L1–L3.
- (26) Tafvizi, A.; Huang, F.; Fersht, A. R.; Mirny, L. A.; van Oijen, A. M. A Single-Molecule Characterization of p53 Search on DNA. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (2), 563–568.
- (27) Gowers, D. M.; Wilson, G. G.; Halford, S. E. Measurement of the Contributions of 1D and 3D Pathways to the Translocation of a Protein along DNA. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (44), 15883–15888.
- (28) Schonhoft, J. D.; Stivers, J. T. Timing Facilitated Site Transfer of an Enzyme on DNA. *Nat. Chem. Biol.* **2012**, *8* (2), 205–10.
- (29) Tafvizi, A.; Mirny, L. A.; van Oijen, A. M. Dancing on DNA: Kinetic Aspects of Search Processes on DNA. *ChemPhysChem* **2011**, *12* (8), 1481–1489.
- (30) Gerland, U.; Moroz, J. D.; Hwa, T. Physical Constraints and Functional Characteristics of Transcription Factor-DNA Interaction. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (19), 12015–12020.
- (31) Benichou, O.; Kafri, Y.; Sheinman, M.; Voituriez, R. Searching Fast for a Target on DNA without Falling to Traps. *Phys. Rev. Lett.* **2009**, *103*, 13.
- (32) Zhou, H. X. Rapid Search for Specific Sites on DNA through Conformational Switch of Nonspecifically Bound Proteins. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (21), 8651–8656.
- (33) Kolomeisky, A.; Veksler, A. Speed-Selectivity Paradox in the Protein Search for Tar Gets on DNA: Is It Real or Not?, in press.

- (34) Zandarashvili, L.; Vuzman, D.; Esadze, A.; Takayama, Y.; Sahu, D.; Levy, Y.; Iwahara, J. Asymmetrical Roles of Zinc Fingers in Dynamic DNA-Scanning Process by the Inducible Transcription Factor Egr-1. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109* (26), E1724–32.
- (35) Iwahara, J.; Levy, Y. Speed-Stability Paradox in DNA-Scanning by Zinc-Finger Proteins. *Transcription* **2013**, *4*, 1–4.
- (36) Marcovitz, A.; Levy, Y. Frustration in Protein-DNA Binding Influences Conformational Switching and Target Search Kinetics. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (44), 17957–17962.
- (37) Pingoud, A.; Wende, W. A Sliding Restriction Enzyme Pauses. *Structure* **2007**, *15* (4), 391–393.
- (38) Shazman, S.; Mandel-Gutfreund, Y. Classifying RNA-Binding Proteins Based on Electrostatic Properties. *PLoS Comput. Biol.* **2008**, *4*, (8).
- (39) Shaul, Y.; Schreiber, G. Exploring the Charge Space of Protein-Protein Association: A Proteomic Study. *Proteins: Struct., Funct., Bioinf.* **2005**, *60* (3), 341–352.
- (40) Bahadur, R. P.; Chakrabarti, P.; Rodier, F.; Janin, J. Dissecting Subunit Interfaces in Homodimeric Proteins. *Proteins: Struct., Funct., Genet.* **2003**, *53* (3), 708–719.
- (41) Lamers, M. H.; Perrakis, A.; Enzlin, J. H.; Winterwerp, H. H. K.; de Wind, N.; Sixma, T. K. The Crystal Structure of DNA Mismatch Repair Protein MutS Binding to a G Center Dot T Mismatch. *Nature* **2000**, *407* (6805), 711–717.
- (42) Wunderlich, Z.; Mirny, L. A. Different Gene Regulation Strategies Revealed by Analysis of Binding Motifs. *Trends Genet.* **2009**, *25* (10), 434–440.
- (43) Mirny, L.; Slutsky, M. How a Protein Searches for Its Site on DNA: the mechanism of facilitated diffusion. *J. Phys. A: Math. Theor.* **2009**, *42*, 434013.
- (44) Zwanzig, R. Diffusion in a Rough Potential. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85* (7), 2029–2030.
- (45) Sun, J.; Viadiu, H.; Aggarwal, A. K.; Weinstein, H. Energetic and Structural Considerations for the Mechanism of Protein Sliding along DNA in the Nonspecific BamHI-Dna Complex. *Biophys. J.* **2003**, *84* (5), 3317–3325.
- (46) Hammar, P.; Leroy, P.; Mahmutovic, A.; Marklund, E. G.; Berg, O. G.; Elf, J. The lac Repressor Displays Facilitated Diffusion in Living Cells. *Science* **2012**, *336* (6088), 1595–1598.
- (47) Sanchez, I. E.; Ferreira, D. U.; Dellarole, M.; de Prat-Gay, G. Experimental Snapshots of a Protein-DNA Binding Landscape. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107* (17), 7751–7756.
- (48) Marcovitz, A.; Levy, Y. Obstacles May Facilitate and Direct DNA Search by Proteins. *Biophys. J.* **2013**, *104* (9), 2042–2050.
- (49) Kaplan, N.; Moore, I. K.; Fondufe-Mittendorf, Y.; Gossett, A. J.; Tillo, D.; Field, Y.; LeProust, E. M.; Hughes, T. R.; Lieb, J. D.; Widom, J.; Segal, E. The DNA-Encoded Nucleosome Organization of a Eukaryotic Genome. *Nature* **2009**, *458* (7236), 362–U129.
- (50) Horton, J. R.; Liebert, K.; Hattman, S.; Jeltsch, A.; Cheng, X. D. Transition from Nonspecific to Specific DNA Interactions along the Substrate-Recognition Pathway of Dam Methyltransferase. *Cell* **2005**, *121* (3), 349–361.
- (51) Bouvier, B.; Zakrzewska, K.; Lavery, R. Protein-DNA Recognition Triggered by a DNA Conformational Switch. *Angew. Chem., Int. Ed.* **2011**, *50* (29), 6516–6518.
- (52) Chen, C. Y.; Pettitt, B. M. The Binding Process of a Nonspecific Enzyme with DNA. *Biophys. J.* **2011**, *101*, 1139–1147.
- (53) Bryngelson, J. D.; Wolynes, P. G. Spin Glasses and the Statistical Mechanics of Protein Folding. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7524–7528.
- (54) Onuchic, J. N.; Wolynes, P. G. Theory of Protein Folding. *Curr. Opin. Struct. Biol.* **2004**, *14* (1), 70–75.
- (55) Yang, S.; Cho, S.; Levy, Y.; Cheung, M. S.; Levine, H.; Wolynes, P. G.; Onuchic, J. N. Domain Swapping is a Consequence of the Principle of Minimal Frustration. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12786–12791.
- (56) Ferreira, D. U.; Hegler, J. A.; Komives, E. A.; Wolynes, P. G. On the Role of Frustration in the Energy Landscapes of Allosteric Proteins. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (9), 3499–503.
- (57) Sutto, L.; Latzer, J.; Hegler, J. A.; Ferreira, D. U.; Wolynes, P. G. Consequences of Localized Frustration for the Folding Mechanism of the IM7 Protein. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (50), 19825–19830.
- (58) Ferreira, D. U.; Hegler, J. A.; Komives, E. A.; Wolynes, P. G. Localizing Frustration in Native Proteins and Protein Assemblies. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (50), 19819–19824.
- (59) Levy, Y.; Cho, S. S.; Shen, T.; Onuchic, J. N.; Wolynes, P. G. Symmetry and Frustration in Protein Energy Landscapes: A Near Degeneracy Resolves the Rop Dimer-Folding Mystery. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 2373–2378.
- (60) Jenik, M.; Parra, R. G.; Radosky, L. G.; Turjanski, A.; Wolynes, P. G.; Ferreira, D. U. Protein Frustratometer: a Tool to Localize Energetic Frustration in Protein Molecules. *Nucleic Acids Res.* **2012**, *40* (Web Server issue), W348–51.
- (61) Zheng, W.; Schafer, N. P.; Wolynes, P. G. Frustration in the Energy Landscapes of Multidomain Protein Misfolding. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110* (5), 1680–1685.
- (62) Clementi, C.; Nymeyer, H.; Onuchic, J. N. Topological and Energetic Factors: What Determines the Structural Details of the Transition State Ensemble and “En-Route” Intermediates for Protein Folding? An Investigation for Small Globular Proteins. *J. Mol. Biol.* **2000**, *298* (5), 937–953.
- (63) Marcovitz, A.; Levy, Y. Arc-Repressor Dimerization on DNA: Folding Rate Enhancement by Colocalization. *Biophys. J.* **2009**, *96* (10), 4212–4220.
- (64) Vuzman, D.; Azia, A.; Levy, Y. Searching DNA via a “Monkey Bar” Mechanism: The Significance of Disordered Tails. *J. Mol. Biol.* **2010**, *396* (3), 674–684.