

# Sequence-dependent sliding kinetics of p53

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**Proper timing of gene expression requires that transcription factors (TFs) efficiently locate and bind their target sites within a genome. Theoretical studies have long proposed that one-dimensional sliding along DNA while simultaneously reading its sequence can accelerate TF's location of target sites. Sliding by prokaryotic and eukaryotic TFs were subsequently observed. More recent theoretical investigations have argued that simultaneous reading and sliding is not possible for TFs without their possessing at least two DNA-binding modes. The tumor suppressor p53 has been shown to slide on DNA, and recent experiments have offered structural and single molecule support for a two-mode model for the protein. If the model is applicable to p53, then the requirement that TFs be able to read while sliding implies that noncognate sites will affect p53's mobility on DNA, which will thus be generally sequence-dependent. Here, we confirm this prediction with single-molecule microscopy measurements of p53's local diffusivity on noncognate DNA. We show how a two-mode model accurately predicts the variation in local diffusivity, while a single-mode model does not. We further determine that the best model of sequence-specific binding energy includes terms for "hemi-specific" binding, with one dimer of tetrameric p53 binding specifically to a half-site and the other binding nonspecifically to noncognate DNA. Our work provides evidence that the recognition by p53 of its targets and the timing thereof can depend on its noncognate binding properties and its ability to change between multiple modes of binding, in addition to the much better-studied effects of cognate-site binding.**

protein-DNA interactions | protein-DNA search | promoter search | energy landscape | one-dimensional diffusion

**T**umor suppressor p53 is known as the "guardian of the genome." The protein is mutated in more than 50% of cancers (1), and plays important roles in activating DNA-repair, cell-cycle arrest, and apoptosis. To prevent the replication of damaged DNA, damage-activated p53 must reach its target promoters sufficiently fast.

In addition to its clinical importance, p53 is the first eukaryotic transcription factor (TF) directly observed to undergo one-dimensional (1D) diffusion on DNA (2). This 1D sliding has long been proposed to facilitate search by DNA-binding proteins (DBPs) (3, 4) and was characterized by single-molecule experiments for RNA polymerase (5, 6), mitochondrial repair enzymes (7), *lac* repressor (8, 9), and repair complex Msh2–Msh6 (10) (see ref. 11 for review). Sliding of p53 was first demonstrated by bulk biochemical experiments and was shown to play a role in p53's activation of target genes (12, 13). Several theoretical and experimental studies (7, 8, 14, 15) have shown that despite a vast excess of accessible DNA ( $10^7$ – $10^9$  bp) to which DBPs have nonspecific affinity, their search process can be efficient if they alternate rounds of 1D sliding while bound nonspecifically with rounds of three-dimensional (3D) diffusion between different sections of DNA. Until recently, *in vivo* studies have been limited to bacterial systems (8), and it remained unclear whether the same mechanism was at play in eukaryotes where DNA is packed by nucleosomes limiting space for sliding. Recently Larson et al. (16) have demonstrated that yeast DBPs search for their sites

by a 1D/3D mechanism. By demonstrating the ability of p53 not only to slide but also to "read" DNA while sliding, our study provides strong support for a 1D/3D mechanism in high eukaryotes. An earlier single-molecule study of Harada et al. (6) found sequence-dependence in the dissociation kinetics of RNA polymerase, but not in its sliding kinetics.

For sliding to be functional in facilitating search, DBPs must be able to read the DNA sequence they slide on. This implies that the binding energy at each DNA position depends on the sequence. The magnitude of this sequence dependence can be captured by the standard deviation of the energies comprising the landscape,  $\sigma$ . Prior theoretical work (17) demonstrated that  $\sigma \lesssim 1.5 k_B T$  is required for fast sliding and facilitated search, while stability of the protein–DNA complex requires  $\sigma \gtrsim 5 k_B T$ . These mutually unsatisfiable requirements lead to an apparent "speed-stability paradox" (17, 18), which had been qualitatively anticipated (19).

A multimode model protein–DNA interaction has been proposed to resolve this paradox (17, 18): In their simplest form, DBPs exhibit two modes of binding and stochastically switch between them: a search (S) mode, and a recognition (R) mode (Fig. 1 *A* and *B*). In S mode, DBPs bind with small sequence-dependence ( $\lesssim 1.5 k_B T$ ) and slide efficiently. In R mode, DBPs bind highly specifically and sliding is negligible. Fast search requires the S mode to have significantly lower average energy and thus be favored at nearly all DNA positions to avoid unproductive visits to R mode (18). Transition into R mode at cognate-like sites that have low energy slows down sliding, but allows recognition of the cognate site. The central idea of this study is that such slowdown on near-cognate sites can be detected experimentally.

Experimental evidence supporting this multimode model includes NMR studies that found specific and nonspecific conformations for *lac* repressor (20, 21) on DNA, and X-ray crystallography of the restriction enzyme BstYI (22). While an NMR study of homeodomain hopping and sliding on DNA found only small structural changes between specific and nonspecific binding, the homeodomain is only weakly specific, with only 200-fold preference for its cognate site over nonspecific DNA (23), compared with p53's  $\sim 10^5$ -fold preference (24). Transcriptional activation by the yeast TF *Mbp1* has been shown to involve 1D sliding (16). Recently, multiple conformations have been identified by electron microscopy for p53–DNA complexes (25). Further support for a multimode search comes from a single-molecule study of p53 truncation mutants showing that distinct

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these areas (Fig. 5A). We demonstrate that  $D/D_0$  is ratio of the time  $t_s$  a protein spends sliding in **S** mode to the total time the protein spend on the landscape,  $t_{\text{total}}$ :

$$\frac{D}{D_0} = \left\langle \frac{t_s}{t_{\text{total}}} \right\rangle = \frac{n \exp(-2E_S)}{\sum_x^n \exp(-U(x))}. \quad [1]$$

This result is based on the assumption that traps are isolated or that the protein does not slide in **R** mode.

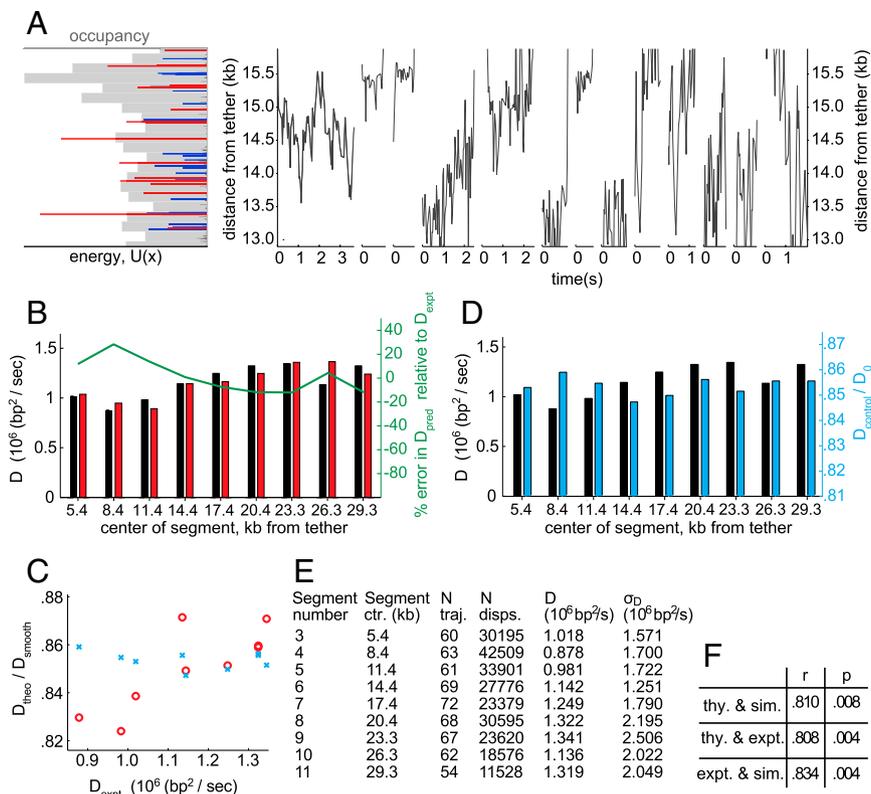
We compared  $D_{\text{expt}}$  with  $D/D_0$  over the segments and found the experimental and predicted diffusion coefficients to correlate strongly ( $r = 0.81, p = 0.008$ ) (Fig. 5B, black and red bars). To assess the significance of this correlation,  $r_{\text{expt}}$ , we constructed 500 random permutations of  $E_R(x)$ , computed  $U(x)$  and  $D/D_0$  for each of them, and determined the resulting  $r_{\text{ctl}}$  between predicted  $D/D_0$  and experimental  $D_{\text{expt}}$  over the segments (example in Fig. 5C and D). The correlation between predicted  $D/D_0$  and  $D_{\text{expt}}$  exceeded that between control  $D/D_0$  and  $D_{\text{expt}}$  for all but 4 of the 500 control landscapes ( $p = 0.008$ ). The observed strong and significant correlation demonstrates that a two-mode sequence-specific landscape can explain the observed positional variability of p53's diffusion coefficient. Summary statistics of the segments'  $D_{\text{expt}}$  are given in Fig. 5E.

We also compared the ruggedness of effective landscape  $U(x)$ , formed from an **R** and an **S** landscape, with earlier experiments and with theoretical requirements. Satisfyingly, the global rug-

gedness  $\sigma$  of the two-mode landscape is  $0.51 k_B T$ , which lies below the theoretical upper limit for efficient search,  $\sim 1.5 k_B T$ , and falls within the uncertainty for the aggregate  $\sigma, 0.84 \pm 0.40 k_B T$  obtained for p53 earlier (2). In contrast, the landscape without a nonspecific binding mode has  $\sigma = 3.5 k_B T$ , which is too great on theoretical grounds for efficient sliding and moreover is incompatible with observed diffusion coefficients. Furthermore, the diffusivity  $D/D_0$  computed for the one-mode landscape shows no significant correlation with  $D_{\text{expt}}$  ( $r = 0.51, p = 0.11$ ), ruling out the one-mode model.

Experimental observations of dimeric p53 binding to half-sites (27) prompted us to explore hemi-specific binding by tetrameric p53. The two-mode model discussed thus far does not require that the two dimers making up the tetramer bind in the same mode. This enhances p53's affinity even to those half-sites that are flanked by sequences that would be unfavorable to bind in **R** mode (Fig. 4D). When we eliminated from our model the hemi-specific mode—represented by the two middle terms of Eq. 4, the correlation between segments'  $D_{\text{expt}}$  and predicted  $D/D_0$  decreased ( $r_{\text{no hemi}} = 0.72$  versus  $r = 0.81$ ). The fraction of the sequence-specificity of p53's diffusion coefficient that owes to full-sites is thus approximately  $(r_{\text{no hemi}}^2 / r^2) = 0.78$ , and the fraction that owes to half-sites is approximately 0.22.

As a further test of the two-mode model, we performed Gillespie simulations (30) of a protein undergoing a random walk on the two-mode landscape. Drift from buffer flow and noise



**Fig. 5.** Comparison of theory, simulations, and experiment. (A) (Right) Random third of trajectories with center in segment 4, ordered by increasing estimated  $D$ . (Left) Predicted potential wells denoted by red (full-sites) and blue (half-sites) bars, with height of bars proportional to predicted effective energy,  $U(x)$ . Gray bars are a histogram of observed occupancy of all particles within the segment, with bin widths equal to one-twentieth the segment width. (B) Estimated  $D$  for experimental (black bars, same as in Fig. 3D) and predicted  $D/D_0$  (red bars) for the predicted landscape, over segments along  $\lambda$  DNA.  $D/D_0$  is scaled to match  $D_{\text{expt}}$ 's mean and coefficient of variation. Green trace is the percent error in predicted  $D/D_0$  normalized by the mean  $D/D_0$ , relative to  $D_{\text{expt}}$  normalized by the mean  $D_{\text{expt}}$ . (C) Scatter plot of  $D_{\text{expt}}$  versus  $D/D_0$  for all segments. Red circles correspond to values for the predicted landscape based on the two-mode model; cyan x's correspond to values for the control landscape whose correlation with  $D_{\text{expt}}$  was the median from among the 500 control landscapes. (D) Black bars are identical to those in B. Cyan bars correspond to  $D/D_0$  of the control landscape that produced the cyan x's in C. (E) Summary statistics of experimental data. For each segment, we show the number of particles contributing to the segment's  $D_{\text{expt}}$ , the number of displacements contributing likewise, the estimates of  $D_{\text{expt}}$  as determined in *Data analysis in Methods*, and the standard deviation of particle's  $D$  weighted by the number of displacements contributed by that particle. (F) Correlation coefficients and  $p$ -values.

from DNA fluctuations were added to the simulated data, which we analyzed identically to the data for experimental  $D$ s. The simulated and experimental diffusion coefficients across the segments correlate strongly ( $r = 0.834$ ,  $p = 0.004$ ) (Fig. 5F). Simulations on the same 500 control landscapes described above were used to determine statistical significance, and thus provide similar validation as do analytical results of the sequence-specific sliding of p53 by the mechanism of two modes of interaction with DNA.

## Discussion

We previously proposed a two-mode model of protein–DNA interaction that allows for fast search and specific binding (17, 18). Our earlier single-molecule measurements of p53 sliding on DNA revealed that the protein slides with sufficiently low friction to satisfy the model’s requirements for efficient search (2). The present study shows that p53 can read the sequence of the DNA on which it is sliding, which is essential for sliding to be functional in accelerating target localization. Our data further suggest that the protein reads, in addition to canonical and near-canonical 20-bp full-sites, half-sites of 10 bp. This conclusion agrees with recent studies showing a role for hemi-specific binding in transcriptional activation at high p53 expression levels (31).

Our results indicate that hemi-specific binding is a general phenomenon of p53–DNA interactions, and not limited to a few known half-site REs. In addition to transcriptional activation, we conjecture that hemi-specific binding might serve to titrate p53 or bias the preactivation distribution of p53 on DNA. This latter function especially is suggested by clustering of degenerate p53 REs near canonical REs, which has also been found for other mammalian TFs (32). Odd numbers of half-sites have been found in many p53 binding sites (33, 34); hemi-specific binding would allow finer tuning of transcriptional activation of p53’s targets.

Our model of p53–DNA binding energy is based on a PWM approximation that was shown to be sound for the four eukaryotic TFs studied (29) as well as for p53 in the case of good full-sites (35), but it omits some observed peculiarities of p53 REs such as gaps within half-sites (33), stronger conservation within a full-site of the first half-site than the second (33), and transcriptional activation from three-quarter sites (36). Accounting for these complexities might yield a stronger correlation between predicted and experimental  $D$ , at the expense of model simplicity.

The two-mode model can be generalized to include transition states or a reaction coordinate of the conformational transition in the protein–DNA complex (17, 18). Molecular dynamics studies of TF–DNA association indeed show a range of conformations (37). Our estimate of  $D/D_0$  is equivalent to the ratio of partition functions of a flat landscape and the predicted “golf-course” landscape. As such, it is independent of the transition rates between **R** and **S** modes, since holding the binding energy of the protein in **R** mode and in **S** mode at a given position constant requires the **R**-to-**S** and **S**-to-**R** rates to vary by the same constant factor. On sufficiently long timescales of sliding, a visit to the **R** state that lasts  $n$  times longer will happen  $n$  times less frequently. And although our multiple landscapes are motivated by our prior work on the global protein–DNA search, they are compatible with other such treatments as well (38, 39).

While enough sites on the  $\lambda$  genome have sufficient affinity for p53 to measurably affect its sliding kinetics, in future work, we intend to add known p53 REs to  $\lambda$  and other DNA (*SI Text, Control for Specific Binding*) to observe stronger sequence-dependent effects, and to answer questions such as whether p53 can slide over a target site without recognizing (binding stably to) it.

We report here the observation of sequence-dependent 1D diffusional kinetics of p53 on DNA. We offer additional experimental support for the importance of 1D diffusion in the kinetics of transcriptional regulation and protein–DNA recognition. With p53 at least, a full understanding of how its complex promoter

architecture functions in transcriptional regulation requires consideration of moves by the protein on DNA even after it has found its cognate site and the ability of the protein to recognize both full- and half-sites while undergoing those moves. Evidence for a multimode model of p53’s binding to DNA suggests that the protein’s function may be disrupted not only by the comparatively well-studied mutations in cognate-site binding residues, but also by mutations that affect its nonspecific interaction with DNA or its ability to transition between specific and nonspecific modes, with potential importance for human health.

## Methods

**Materials and Data Acquisition.** The optical setup, DNA constructs, labeled p53, and flow cells (Fig. 2A) were as described previously (2), excepting that the protein was labeled with AlexaFluor 555 (Invitrogen) and illuminated by the 532 nm line of a Nd:YAG laser, and that fiducial beads were used to align p53 movies and DNA movies (*SI Text, Materials and Data Acquisition*).

**Data Analysis.** Protein molecules were assigned to individual DNA molecules, and their trajectories were recorded using scripts written in MATLAB (MathWorks). Positions of the p53 molecules in space along the DNA image were mapped to positions on the contour of the DNA using Brownian dynamics simulations (*SI Text, Data Analysis*). Fig. 2C shows three sample trajectories.

We determined a diffusion coefficient  $D$  for every trajectory recorded except for those of particles stuck to the flow-cell surface (*SI Text, Materials and Data Acquisition*). We used maximum likelihood estimation, correcting for biased drift from buffer flow as well as for fluctuation in the  $\lambda$ -phage DNA on which p53 diffused. We found  $D$  for a particle to be estimated by

$$D = \frac{1}{2} \left( \frac{1}{n} \sum_i \frac{(\Delta x_i - v \Delta t_i)^2}{\Delta t_i} - \frac{1}{n} \sum_{\Delta t} n_{\Delta t} \frac{\langle \Delta x_{d,\Delta t}^2 \rangle}{\Delta t} \right), \quad [2]$$

where  $v$  is the drift velocity (*SI Text, Eq. S3* in *Data Analysis*).

An  $N$ -frame trajectory contains  $(N-1)(N-2)/2 \equiv n$  displacements. The  $i$ th observed displacements in space and in time are, respectively,  $\Delta x_i$  and  $\Delta t_i$ . The second sum in Eq. 2 is over time windows  $\Delta t$  ranging from the camera frame rate, 30 ms, to 2 s. The quantity under the sum is the mean squared displacement of the DNA itself owing to Brownian fluctuations,  $\langle \Delta x_{d,\Delta t}^2 \rangle$  on a timescale of  $\Delta t$ , as calculated from measurements of quantum dots covalently attached to the DNA (*SI Text, Interpolations of DNA-Fluctuation Variance and Distributions*), divided by  $\Delta t$ , and weighted by the number of displacements with a corresponding  $\Delta t$ . The first sum represents the apparent diffusion coefficient of p53, corrected for drift.  $D_{\text{expt}}$  for a segment is the average of all particles in the segment, weighted by the number of displacements contributed.

**Prediction of Diffusion Coefficients.** In its most basic form, our two-mode model posits a recognition (**R**) mode in which a protein’s binding energy,  $E_R$ , is dependent on its position on DNA,  $x$ , and a search (**S**) mode in which its binding energy,  $E_S$ , is constant.

To determine  $E_R(x)$ , we scored the  $\lambda$  genome using position weight matrices ( $PWM_S$ ) of p53 half-sites derived from a catalogue of p53 binding sites (28) (Fig. 4A). We assume that differences between scores are proportional to differences between corresponding half-site energies:

$$E_R(x) - E_S = c(PWM(x) - PWM_S), \quad [3]$$

where  $PWM(x)$  is the score for position  $x$ , and  $PWM_S$  is the score corresponding to binding energy in the **S** mode, and  $c$  is a conversion factor between score and energy (in  $k_B T$ ). Since p53 is a dimer of dimers with each dimer able to bind independently to a 10-bp half-site (27). For binding in **R** mode, then, the left dimer binds with energy  $E_R(x)$  and the right dimer binds with energy  $E_R(x + \Delta)$ . The statistical weight  $w(x)$  of a site  $x$  is thus the sum of the Boltzmann factors corresponding to each of the four modes, and the negative logarithm of  $w(x)$  is the effective binding energy of p53 to  $\lambda$  DNA at position  $x$ :

$$U(x) = -\log w(x);$$

$$w(x) = e^{-2E_s} + e^{-(E_s+E_R(x+\Delta))} + e^{-(E_R(x)+E_s)} + e^{-(E_R(x)+E_R(x+\Delta)+\epsilon)}, \quad [4]$$

with the spacing between the 5' ends of half-sites,  $\Delta$ , usually a half-site length, 10 bp. It can be greater for full-sites with a 1–14 bp gap, which we account for as described in *SI Text, Prediction of Energy Landscape and Local Diffusion Coefficients*.

Three physical parameters relate *PWM* scores to the effective landscape  $U(x)$ :  $PWM_5$  and  $c$ , from Eq. 3, and the cooperativity term  $\epsilon$ , which represents additional binding energy when both dimers are bound in **R** mode. All three parameters are taken from bulk biochemical data (24, 29), described in *SI Text, Prediction of Energy Landscape and Local Diffusion Coefficients*.

The effective landscape in Eq. 4 was used to predict local  $D$  of p53 by calculating over a segment's  $n$  positions the reduction in diffusivity owing to sequence-specific binding as compared to a featureless landscape,  $D/D_0$ , as

$$\frac{D}{D_0} = \left\langle \frac{t_s}{t_{\text{total}}} \right\rangle = \frac{1}{\frac{1}{n} \sum_x^n \exp(-U(x))}. \quad [5]$$

As described in *SI Text, Prediction of Energy Landscape and Local Diffusion Coefficients*, we found that  $D/D_0$  in a segment should equal the mean fraction of time a particle in that segment spends sliding,  $t_s/t_{\text{total}}$ , which in turn equals the reciprocal of the average statistical weight [equivalent to  $\exp(-U(x))$ ] over all sites in the segment of Eq. 4. We assume that microscopic step rates to positions  $x-1$  and  $x+1$  are equal.

Once we computed  $D/D_0$  for each segment, we made a correction to account for uncertainty in the assignment of experimental displacements to segments owing to DNA fluctuations, described in *SI Text, Interpolations of DNA-Fluctuation Variance and Distributions*. We then assessed the quality of our predicted diffusion coefficients by computing Pearson's correlation coefficient  $r_{\text{expt}}$  between experimental diffusion coefficients  $D_{\text{expt}}$  and predicted  $D/D_0$  over the segments, and determined statistical significance of the correlation using a permutation test described in *SI Text, Prediction of Energy Landscape and Local Diffusion Coefficients*.

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