Supporting Information

for

Facilitated Dissociation of Transcription Factors from Single DNA Binding Sites

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S1. Materials and Methods

A. Protein purification

Expression and purification of wtFis, gfpFis, wtNHP6A, and NHP6Agfp, all without tags, have been described previously (13, 43, 44).

B. DNA binding sites

Binding sites for protein molecules consist of Cy3-labeled and biotinylated dsDNA molecules purchased from Integrated DNA Technology (IDT). For Fis, we use a sequence called F1 (21) which is formed by annealing a forward strand: 5'-AAA TTT GCT CAA AAT TCA AAC AAA TTT-Cy3-3' to a complementary reverse strand: 5'-/5AmMC6/AAA TTT GTT TGA ATT TTG AGC AAA TTT-biotin-3' (/5AmMC6/ refers to a 5' amino modifier from IDT) in annealing buffer (10mM Tris, pH 8.0, 1mM EDTA and 50mM NaCl in nuclease-free water). Annealed, Cy3-labeled dsDNA is diluted into annealing buffer supplemented with 0.2% Triton-X100 and 0.5 mg/mL casein to prevent DNA from sticking to storage tubes.

Binding sites for NHP6A molecules also consist of Cy3-labeled dsDNA, but contain the recognition sequence for the human sex-determining region Y protein SRY which also functions as a target for NHP6A binding (9)[change ref. to Masse]. The forward strand is: 5'-/5AmMC6/AAA TTT GTG AAT GTT CAA AAT TTG G-biotin-3' and the reverse strand is 5'-CCA AAT TTT GAA CAA TCA CAA AAT T-Cy3-3'. Annealing and storage are done in the same buffers used for F1 sequences. We checked that DNA strands annealed together properly using gel electrophoresis.

C. Flow cell preparation and imaging buffer

Flow cells are made by sandwiching thin strips of double-sided adhesive tape between rectangular borosilicate glass coverslips (Fisher Scientific, catalog number: 12-544-E, size: 24X50mm, No. 1.5) and 1"X3" glass slides. The volume of a flow cell is approximately 10μL. Glass slides have holes drilled on both ends of the flow cells to allow for buffer exchange using a pipette. Slides and coverslips are cleaned and functionalized using methods adapted from previously described procedures (45). Slides and coverslips are amino-modified using amino silane (N-(2-aminoethyl)-3-aminopropyltrimethoxysilane; United Chemical Technologies). Slides and coverslips are PEGylated for 2 hours, or overnight, using mPEG (mPEG-SVA-5000, Laysan Bio) in 5.4 mg/mL sodium bicarbonate solution, however coverslips use an additional 4-7% of biotinylated PEG (Biotin-PEG-SVA-5000, Laysan Bio) to allow attachment of biotinylated DNA to the surface via biotin-streptavidin linkage.

The buffer used for imaging, rinsing flow cells, and diluting proteins (referred to as T-X buffer, where X is between 10 and 300 depending on the NaCl concentration used in the experiment) contains X mM NaCl, 3 mM $MgCl_2$, and 10 mM Tris-HCl (adjusted to pH 7.55±0.04 using NaOH) in nuclease-free water. Flow cells are incubated with 0.5 mg/mL casein (Sigma Aldrich) to prevent non-specific adhesion of biomolecules. Next, flow cells are incubated with 0.2 mg/mL streptavidin (Invitrogen), followed by incubation with 2-20 pM of either F1 or SRY Cy3-labeled dsDNA. Flow cells are then re-incubated with casein before addition of ~3nM gfpFis or ~30nM NHP6Agfp. All incubations are for 5

min and contain 0.5 mg/mL casein. Flow cells are rinsed with 140 μL of T-X buffer between all incubations.

Protein-free imaging buffer (made from T-X buffer) contains 1% βME, 0.5 mg/mL casein, and an oxygen scavenging system consisting of 0.04 mg/mL catalase (Sigma Aldrich), 0.1 mg/mL pyranose oxidase (Sigma Aldrich), and 0.4% D-glucose. We used pyranose oxidase instead of glucose oxidase to eliminate acidification of the imaging buffer (46). For protein competition experiments, gfpFis or NHP6Agfp were added to imaging buffer at the desired concentration by diluting from glycerol-containing stocks stored at -20°C.

D. Single molecule fluorescence microscopy

Prepared flow cells were imaged at room temperature using objective-type total internal reflection fluorescence (TIRF) microscopy on an inverted microscope (IX81, Olympus). Cy3-DNA molecules and gfp fusion proteins were excited using the evanescent waves from fiber-coupled lasers with wavelengths of 561 nm (85 YCA 075-115, Melles Griot) and 488 nm (Sapphire 488-20, Coherent), respectively. Prior to placing lasers in TIR configuration, we measure the laser power exiting the objective to be 10 mW (561 nm) and 1.6 mW (488 nm). Fluorescence from both molecules is collected using a 100X, 1.45NA oil objective and is filtered using a dual-laser filter set (U-Nz488/561, Chroma Technology). Cy3 and GFP fluorescence is spectrally separated into different channels by temporally separating the excitation pulses of each laser using computer controlled shutters on each laser.

The timing sequence for each survival fraction measurement consists of either a 10 or 15 frame image stack of a single region on the flow cell. Each Cy3 and GFP channel exposure is, respectively, 350 ms and 500 ms with a 37 ms lag between the end of the Cy3 exposure and the beginning of the GFP exposure. The repetition time is 1 second, so a survival fraction measurement takes approximately 10 seconds or 15 seconds depending on whether 10 or 15 frame image stacks were used. Subsequent measurements (i.e. subsequent image stacks) are taken from regions of the flow cell that have not been previously exposed to excitation light (Fig 1*A*, Main Text). This ensures that photobleaching does not affect our survival fraction measurements since each field-of-view receives equal doses of light energy, and also ensures that our experiment is insensitive to drift. The fluorescence emission, which appears as diffraction-limited signals (~320 nm FWHM), is collected onto the 512X512 pixel array of a force-air-cooled EMCCD camera (Hamamatsu) and read out for storage onto a computer.

E. Single molecule fluorescence image processing

Processing of image stacks is accomplished using an analysis pipeline built from homemade software in MATLAB (MathWorks). For each channel in an image stack, the positions (x_o, y_o) of the signals in each frame were determined by fitting the signal profile to a two-dimensional Gaussian,

$$\Psi(x,y) = \frac{4I_o \ln 2}{\pi s^2} \exp\left\{\frac{-4\ln 2}{s^2}\left((x-x_o)^2 + (y-y_o)^2\right)\right\} + b, \qquad [S1]$$

where I_o is the signal intensity, s is the full-width at half-maximum, and b is the background level. The locations of the signals in the gfpFis channel are correlated between frames to generate trajectories. Counting the number of trajectories provides the number of detected gfpFis molecules.

Trajectories in the gfpFis channel are selected for final analysis based on whether they spatially co-localize (i.e. whether they are within 0.55 pixels in both the x and y directions) with a DNA signal in the Cy3 channel. This serves as a quality control to ensure that only gfpFis molecules bound to Cy3-labeled DNA binding sites are retained for analysis. We checked that off-resonant excitation of Cy3(GFP) does not register in the GFP(Cy3) channel.

The survival fraction S_i at the ith time-point is determined by first counting the number of co-localized gfpFis signals G_i in the ith image stack. To divide out any variation of the number of Cy3 signals along the length of the flow cell, G_i is divided by the average number of Cy3-DNA signals R_i in the image stack. The survival fraction is finally given by

$$S_\mathrm{i} = \frac{G_\mathrm{i}}{R_\mathrm{i} A_\mathrm{o}} \ , \qquad \text{[S2]}$$

where A_o is an estimate of $G_\mathrm{i}/R_\mathrm{i}$ for the initial image stack (i=0) which is obtained from a fit of $G_\mathrm{i}/R_\mathrm{i}$ to an exponential $A = A_\mathrm{o} \exp(-t_\mathrm{i}/T)$, and where t_i is the time corresponding to the ith image stack.

F. Statistical uncertainty in survival fraction

Error bars on survival fraction decay measurements are estimated by propagating statistical errors in G_i, R_i, and A_o to S_i. The error in G_i is due mainly to counting fluctuations: $\sigma_{G_\mathrm{i}} = \sqrt{G_\mathrm{i}}$. The error in R_i, σ_{R_i}, is given by the standard deviation of the number of Cy3 signals in the image stack. Error in A_o is given by $\sigma_{A_\mathrm{o}} \approx \frac{1}{R_\mathrm{o}} \sqrt{G_\mathrm{o} + \left(\frac{G_\mathrm{o}}{R_\mathrm{o}}\right)^2 \sigma_{R_\mathrm{o}}^2}$, where R_o and σ_{R_o} are, respectively, the average number and standard deviation of Cy3 signals in the initial (i.e. i=0) image stack, and G_o is the number of colocalized gfpFis signals in the same image stack.

G. Determination of off-rate from decay curves

To obtain an estimate of the off-rate k_off from a single measurement, each survival probability curve is fit to

$$S(t) = \exp(-t/\tau_\mathrm{off}), \qquad \text{[S3]}$$

where the off-rate is given by $k_\mathrm{off} = 1/\tau_\mathrm{off}$. Off-rates plotted in Fig. 2*B* (*Main Text*) are averages of individual off-rate measurements,

$$\bar{k}_\mathrm{off} = \frac{\sum_\mathrm{j} \frac{k_\mathrm{off,j}}{\sigma_\mathrm{j}^2}}{\sum_\mathrm{j} \frac{1}{\sigma_\mathrm{j}^2}} \qquad \text{[S4]}$$

weighted by the uncertainty σ_j of each measurement, where the subscript j has been added to run over the number of measurements. Estimates of the uncertainty in the mean off-rate (error bars in Fig. 2*B*) are given by the square-root of an unbiased estimate of the variance:

$$\sigma_k = \sqrt{\frac{\left(\sum_j \frac{1}{\sigma_j^2}\right)\left(\sum_j \frac{\left(k_{off,j} - \bar{k}_{off}\right)^2}{\sigma_j^2}\right)}{\left(\sum_j \frac{1}{\sigma_j^2}\right)^2 - \sum_j \left(\frac{1}{\sigma_j^2}\right)^2}}. \quad [S5]$$

S2. F1 sequences are binding sites for single gfpFis dimers

A. Bleaching-step histograms of gfpFis fluorescence trajectories

To confirm that Cy3-F1 DNA binding sites only accommodate the stable binding of single gfpFis dimers, we recorded fluorescence trajectories of gfpFis molecules bound to F1 binding sites, in protein-free buffer, and counted the number of bleaching-steps until the signals completely bleached. Signals were selected only if they completely bleached at some point their trajectory. Representative gfpFis trajectories displaying one, two, and three bleaching-steps are shown in Fig. S1*A*. Since each subunit of a gfpFis dimer contains a GFP fusion, we expect to see no more than two bleaching-steps per gfpFis signal. A histogram of the number of bleaching-steps is shown in Fig. S1*B* showing that the majority bleached in one or two steps as expected. A minority of trajectories (three out of 50 trajectories, or 6 ± 3 %) were observed to bleach with three bleaching-steps, which we attribute to the occurrence of multiple binding sites being spatially located in diffraction limited areas due to streptavidin multivalency and the likelihood that multiple streptavidins are colocalized within diffraction limited areas (as described below).

B. Estimate of Cy3-F1 binding site spatial density

We estimated the number of biotinylated Cy3-F1 DNA sequences that get distributed among the available surface-bound streptavidin molecules. To do this we first summed the total signal ($4.7\text{X}10^9$ counts/350 ms) contained on the whole 512X512 array of the first frame from the Cy3 channel of the same image stack used to obtain the gfpFis bleaching-step data above. To estimate the fluorescence due only to Cy3 molecules, we subtracted the estimated background level from the total signal to obtain a total Cy3 fluorescence level $\langle F_{\text{Cy3}} \rangle$ of $6.5\text{X}10^8$ counts/350 ms. The background level per pixel was estimated by averaging measurements of the camera counts in selected sub-regions of varying size that were uniformly distributed throughout the image and that were devoid of Cy3-DNA signals. We also recorded fluorescence trajectories of individual Cy3 signals in the same image stack. We only recorded data from Cy3 signals that completely bleached. We estimated the mean brightness $\langle B_{\text{sm}} \rangle$ of a single Cy3 emitter by making a histogram of the sizes of the bleach-steps in the measured trajectories (Fig. S1*C*). The mean brightness of a Cy3-F1 DNA is 141700 ± 9800 counts/signal/ 350 ms . Finally, the total density of Cy3-F1 DNA binding sites is given by $\eta_{\text{Cy3}} = \langle F_{\text{Cy3}} \rangle / \langle B_{\text{sm}} \rangle A = 1.66$ Cy3 molecules $\cdot\ \mu\text{m}^{-2}$ where A is the area of the full 512X512 image.

C. Estimation of streptavidin spatial density

We made estimates of the spatial density of streptavidin in our flow cells as follows. A flow cell, which was exposed to the same amount of streptavidin used in our off-rate measurements, was filled

multiple times with an excess of biotinylated Cy3-DNA (~100 pM), to saturate all available biotin-binding pockets on the streptavidin molecules, and washed with buffer. We measured the total fluorescence contained within a 350 X57 pixel^2 region receiving uniform illumination by the laser field. To prevent saturation of the camera by Cy3-DNA fluorescence, we reduced the laser power to 0.4 mW, reduced the camera integration time for the Cy3 channel to 200 ms, and modified the imaging mode of the camera. Under these conditions, in which surface-bound streptavidin molecules are saturated with biotinylated Cy3-DNA, the images no longer contained individually resolvable diffraction-limited signals, but instead contained a dense lawn of fluorescence. A separate region of the flow cell was exposed to a high laser power (~10 mW) in order to bleach the Cy3-DNA molecules to a level where individual diffraction-limited signals are resolvable. We then reduced the laser power back to 0.4 mW and, using the same camera settings as used in the previous image containing a high density of fluorescence, we recorded fluorescence trajectories of 14 signals from this region, choosing only signals that underwent single-step bleaching. We determined that the mean brightness of a Cy3-DNA signal under these imaging conditions is 55000±5000 counts/signal/200 ms. Taking the ratio of the background-corrected fluorescence in the 350X57 pixel^2 region to the mean brightness of a Cy3-DNA signal gives an estimate for the total number of Cy3 emitters in the same region. Since surface-bound streptavidin has three remaining binding pockets for biotin, we divided the total number of emitters by 3 to obtain an estimate for the total number of streptavidin in the 350X57 pixel^2 region. As a result, we estimate that the functionalized surfaces of our flow cells contain a streptavidin surface density of $\eta_{\mathrm{strep}} \approx 18$ streptavidin $\cdot\ \mu\mathrm{m}^{-2}$.

D. Multivalency and high spatial density of streptavidin explains minority of gfpFis trajectories that bleach in three steps

To determine the expected statistical distribution with which streptavidin biotin-binding pockets are occupied with biotinylated Cy3-DNA molecules, we simulated the process using homemade software in C++. We randomly distributed objects, representing biotinylated Cy3-DNA molecules, into trivalent boxes, representing surface bound streptavidin, and counted the number of streptavidin molecules that were occupied with $\sigma = 0$, 1, 2, or 3 objects. This gives the probability $p(\sigma)$ that any given streptavidin has σ Cy3-DNA binding sites attached to it, which depends on the relative spatial densities of Cy3-DNA molecules η_{Cy3} and streptavidin molecules η_{strep} in a given area. Using the above measured estimates for η_{Cy3} and η_{strep}, we calculated $p(\sigma)$ giving 91.3, 8.3, 0.38, and 0.008 % for $\sigma = 0$, 1, 2, and 3, respectively. We find that, although the absolute probability for any given streptavidin to be doubly occupied by Cy3-DNA is only 0.5 %, this indicates that of all streptavidin molecules that are occupied by at least one binding site, 4.4% of them should contain two binding sites.

We also considered that at a density of $\eta_{\mathrm{strep}} \approx 18$ streptavidin $\cdot\ \mu\mathrm{m}^{-2}$, we should expect a sizable probability for multiple streptavidin molecules to co-localize to a diffraction limited area. The mean number ν of streptavidin per diffraction limited area is given by $\nu = \eta_{\mathrm{strep}}\pi\left(\frac{d}{2}\right)^2$, where d is the diameter of a diffraction limited region which we take to be 250 nm, and gives $\nu = 0.9$. The probability to observe n_{s} randomly distributed streptavidin colocalized to a diffraction-limited region is given by a Poisson distribution $C(n_{\mathrm{s}}) = \frac{\nu^{n_{\mathrm{s}}}e^{-\nu}}{n_{\mathrm{s}}!}$ (47). Including this effect, we constructed the total

probability $P(n)$ to observe n co-localized binding sites in a diffraction-limited area. This probability, normalized to the probability to observe at least one binding site, is shown in Fig. S1*D*. We see that the overall probability to observe two binding sites in a diffraction limited area is $= \frac{P(2)}{P(1)+P(2)+P(3)+P(4)} = 7.6$ %, in excellent agreement with the observed fraction of gfpFis trajectories that underwent three bleach-steps (6 ± 3 %). We conclude that our data is consistent with Cy3-labeled F1 sequences allowing only one gfpFis dimer to stably bind at a time.

S3. Simple model of facilitated dissociation and predicted salt-dependence

The basic kinetic scheme for a model of facilitated dissociation that we use throughout this work is given by

$$\begin{array}{ccccc} 0 & \leftrightarrows & 1 & \leftrightarrows & 2 \\ & & \downarrow & \swarrow & \\ & & 3 & & \end{array} \qquad [S6]$$

A simple schematic version of this model, depicted in Fig. S2*A*, treats each protein molecule as a dimer of identical subunits and was theoretically studied in previous work (27). The DNA binding site is also represented by a dimer of identical subunits. Each protein subunit binds a DNA binding site subunit with binding energy ΔE_{o}. There are two pathways to dissociation. The first is a spontaneous dissociation pathway $(0 \rightarrow 1 \rightarrow 3)$ in which the protein unbinds in two steps. This pathway is independent of proteins in solution. The second pathway $(0 \rightarrow 1 \rightarrow 2 \rightarrow 3)$ requires competitor proteins in solution and involves the invasion of a partially dissociated protein by a competitor leading to facilitated dissociation. *While we employ the same basic kinetic scheme in this work, we do not make the simplifying assumption, made in (27), that the protein unbinds DNA in a symmetric fashion. However, this simplified model is nonetheless useful in qualitatively demonstrating how the partial unbinding hypothesis predicts a weaker salt-dependence when protein molecules are in solution*.

The salt dependence of this type of kinetic pathway, restricted to the special case where the protein unbinds symmetrically from the DNA binding site due to its two-fold symmetry, is derived as follows. The off-rate in this model can be estimated to be (27):

$$k_{\mathrm{off}} \propto e^{2\Delta E_{\mathrm{o}}/k_{\mathrm{B}}T} + ce^{(\Delta E_{\mathrm{o}}+\mu_{\mathrm{o}})/k_{\mathrm{B}}T}. \qquad [S7]$$

Here, ΔE_{o} is the binding energy for a subunit, μ_{o} is a constant reference chemical potential, T is the absolute temperature, and k_{B} is Boltzmann's constant. The first term corresponds to the spontaneous off-rate and the second to the parallel contribution of the protein concentration-dependent pathway, valid for low concentration. The binding energy and dissociation constant, K_{D}, of a subunit are related by:

$$\Delta E_{\mathrm{o}} - \mu_{\mathrm{o}} = k_{\mathrm{B}}T \log K_{\mathrm{D}}. \qquad [S8]$$

Combining Eq. S8 with Eq. 3 (*Main Text*) gives an expression for the salt dependence of the binding energy, $\Delta E_{\mathrm{o}} \propto n \log c_{\mathrm{S}}$, which, when inserted into Eq. S7, provides a prediction for the combined

protein and salt dependence of the off-rate $k_{\mathrm{off}} \propto c_S^{2n} + c_S^n c$. The power-law terms on the right hand side are related to each other by unknown constants that should be set by experimental data. Here, n is interpreted as the number of counterions released when one Fis subunit binds a DNA subunit. Therefore, the partial dissociation model makes the prediction (depicted in Fig. S2*B*) that over some range of c_S, which depends on the protein concentration, the salt dependence of the off-rate is weaker when proteins are in solution. Specifically, in this simple version of the model, the prediction is that the exponent of the power-law is reduced by a factor of two, reflecting the assumption that the partially dissociated state corresponds to loss of exactly half the contacts of the fully-bound state. However, the model that we consider in this work does not assume this symmetry and will in general allow the slope $\partial \log k_{\mathrm{off}} / \partial \log c_S$ to be less than half as large during facilitated dissociation as it is during spontaneous dissociation.

S4. Derivation of mean time to dissociation

The basic kinetic scheme considered throughout this work corresponds to the reaction in Eq. S6 which, in turn, corresponds to the following set of coupled ODEs:

$$\begin{aligned}
\dot{p}_0 &= -k_{01}p_0 + k_{10}p_1 \\
\dot{p}_1 &= k_{01}p_0 - (k_{10} + k_{12} + k_{13})p_1 + k_{21}p_2 \\
\dot{p}_2 &= k_{12}p_1 - (k_{21} + k_{23})p_2 \\
\dot{p}_3 &= k_{13}p_1 + k_{23}p_2
\end{aligned} \qquad \text{[S9]}$$

where we assume that $k_{12} = \gamma c$, and where γ is the bimolecular on-rate constant and c is the concentration of competitors in solution. This system is difficult to solve for arbitrary initial conditions. Instead, a method for exactly calculating the mean reaction time for an arbitrary reaction with an irreversible final step (48) is used to calculate the mean time $\langle \tau_{\mathrm{off}} \rangle$ to transition from state 0 to state 3. We find that k_{off} is given by

$$\langle \tau_{\mathrm{off}} \rangle^{-1} = \frac{k_{01}(k_{12}k_{23} + k_{13}(k_{21} + k_{23}))}{k_{10}(k_{21} + k_{23}) + k_{01}(k_{12} + k_{21} + k_{23}) + k_{12}k_{23} + k_{13}k_{21} + k_{13}k_{23}}. \qquad \text{[S10]}$$

This expression can be written as $k_{\mathrm{off}} = \frac{D + c}{Ac + B}$, with $A = \frac{k_{01} + k_{23}}{k_{01}k_{23}}$, $B = \frac{(k_{01} + k_{10} + k_{13})(k_{21} + k_{23})}{\gamma k_{01}k_{23}}$, and $D = \frac{k_{13}(k_{21} + k_{23})}{\gamma k_{23}}$, and appears as Eq. 1 in the *Main Text*.

S5. Derivation of modified model and associated salt dependence

As outlined in the *Main Text*, we generalized the model of facilitated dissociation by introducing multivalency in the binding of TFs to DNA and by explicitly including salt ions that can compete with TFs for condensation onto DNA (Fig. 4*A*, *Main Text* and Fig. S3). To derive expressions for each of the microscopic kinetic rates k_{ij} in Eq. S10, we impose detailed balance at each kinetic transition along the reaction coordinate in Fig. S3. We assume each kinetic rate has the form

$$k_{\mathrm{ij}} = \nu_{\mathrm{ij}} \exp(-\Delta E^{\mathrm{ij}}_{\mathrm{barrier}}/k_{\mathrm{B}}T) \quad [S11]$$

where ν_{ij} is an attempt rate and $\Delta E^{\mathrm{ij}}_{\mathrm{barrier}}$ is the barrier height going from state i to state j. Between states 0 and 1, we have

$$\frac{k_{01}}{k_{10}} = \frac{\nu_{01}\exp(-\Delta E^{01}_{\mathrm{UB}}/k_{\mathrm{B}}T)}{\nu_{10}\exp(-\Delta E^{10}_{\mathrm{B}}/k_{\mathrm{B}}T)} \equiv \exp(-\Delta G^{01}_{\mathrm{int}}/k_{\mathrm{B}}T) \quad [S12]$$

where $\Delta G^{01}_{\mathrm{int}}$ is the interaction free energy between states 0 and 1. This gives $\Delta G^{01}_{\mathrm{int}} = \Delta E^{01}_{\mathrm{UB}} - \Delta E^{10}_{\mathrm{B}} - k_{\mathrm{B}}T \ln(\frac{\nu_{01}}{\nu_{10}})$. Now we consider that there are salt ions at concentration c_{s} which can condense onto the partially exposed DNA binding site and lower the total free energy $\Delta G^{01}_{\mathrm{total}}$. We have $\Delta G^{01}_{\mathrm{total}} = \Delta G^{01}_{\mathrm{int}} - n_{01}k_{\mathrm{B}}T \ln(c_{\mathrm{s}}/K_{\mathrm{s}})$ where n_{01} is the number of ions that bind the DNA when the TF partially unbinds (i.e. goes from state 0 to 1) and K_{s} is the dissociation constant of a salt ion binding a DNA subunit. Again imposing detailed balance, this time including salt effects, we have

$$\frac{k_{01}}{k_{10}} \equiv \exp\left(-\frac{\Delta G^{01}_{\mathrm{total}}}{k_{\mathrm{B}}T}\right). \quad [S13]$$

At this point we must choose how to distribute salt dependence between k_{01} and k_{10} subject to the constraint imposed by Eq. S13. Throughout this work, we make the assumption that all the salt dependence enters on kinetic steps where salt ions bind to DNA. This results in the following salt dependence for k_{10} and k_{01}

$$k_{10} = \nu_{10} \exp\left(-\frac{\Delta E^{10}_{\mathrm{B}}}{k_{\mathrm{B}}T}\right)$$
$$k_{01} = \nu_{01} \exp\left(-\frac{\Delta E^{01}_{\mathrm{UB}}}{k_{\mathrm{B}}T}\right)\left(\frac{c_{\mathrm{s}}}{K_{\mathrm{s}}}\right)^{n_{01}}. \quad [S14]$$

Next we consider the kinetic steps between states 1 and 2 which depend on the protein concentration c. We have,

$$\frac{k_{12}}{k_{21}} = \frac{\Gamma\exp(-\Delta E^{12}_{\mathrm{B}}/k_{\mathrm{B}}T)\, c}{\nu_{21}\exp(-\Delta E^{21}_{\mathrm{UB}}/k_{\mathrm{B}}T)} \equiv \exp(-\Delta G^{12}_{\mathrm{int}}/k_{\mathrm{B}}T) \quad [S15]$$

where Γ is the diffusion limited attempt rate for proteins in solution to bind to an exposed binding site, and where we identify $\Gamma\exp(-\Delta E^{12}_{\mathrm{B}}/k_{\mathrm{B}}T)$ with the bimolecular on-rate constant γ. This gives $\Delta G^{12}_{\mathrm{int}} = \Delta E^{12}_{\mathrm{B}} - \Delta E^{21}_{\mathrm{UB}} - k_{\mathrm{B}}T \ln(\frac{Gc}{\nu_{21}})$. When the protein from solution binds, it releases the same n_{01} counterions that bound between steps 0 and 1. This increases the free energy giving a total free energy change of $\Delta G^{12}_{\mathrm{total}} = \Delta G^{12}_{\mathrm{int}} + n_{01}k_{\mathrm{B}}T \ln(c_{\mathrm{s}}/K_{\mathrm{s}})$. Again imposing detailed balance, and choosing to put the salt dependence on k_{21} since that is the step where salt ions bind, we get for k_{12} and k_{21}

$$k_{12} = \Gamma \exp\left(-\frac{\Delta E^{12}_{\mathrm{B}}}{k_{\mathrm{B}}T}\right) c = \gamma c$$
$$k_{21} = \nu_{21} \exp\left(-\frac{\Delta E^{21}_{\mathrm{UB}}}{k_{\mathrm{B}}T}\right)\left(\frac{c_{\mathrm{s}}}{K_{\mathrm{s}}}\right)^{n_{01}}. \quad [S16]$$

An analogous procedure is used to derive the salt dependence for rates k_{13} and k_{23}, except that in these cases the corresponding reverse rates, k_{31} and k_{32} respectively, are auxiliary rates used only for calculating k_{13} and k_{23}, and do not enter the model. We obtain

$$k_{13} = \nu_{13} \exp\left(-\frac{\Delta E_{\mathrm{UB}}^{13}}{k_{\mathrm{B}}T}\right)\left(\frac{c_{\mathrm{s}}}{K_{\mathrm{s}}}\right)^{n_{13}}$$
$$k_{23} = \nu_{23} \exp\left(-\frac{\Delta E_{\mathrm{UB}}^{23}}{k_{\mathrm{B}}T}\right)\left(\frac{c_{\mathrm{s}}}{K_{\mathrm{s}}}\right)^{n_{01}+n_{13}}. \qquad [S17]$$

We identify $n_{01} + n_{13}$ with the total number of counterions that can condense along the DNA binding site. Plugging Eqs. S14, S16, and S17 into Eq. S10 gives an expression for the off-rate, $k_{\mathrm{off}} = k_{\mathrm{off}}(c, c_{\mathrm{s}}; \vec{p}_{\mathrm{phys}})$, in terms of the set of 15 physical parameters $\vec{p}_{\mathrm{phys}}$ and the independent experimental control variables (i.e. protein concentration, c, and salt concentration, c_{s}). The set of physical parameters includes 6 attempt rates, six barrier heights, n_{01}, n_{13}, and K_{s}.

S6. Measurement of bimolecular on-rate constant γ

To reduce the number of free parameters in fitting, we directly measured the bimolecular on-rate constant γ. A prepared flow cell was allowed to incubate with 8 pM of Cy3-F1 DNA binding sites for 5 min, and excess DNA washed away with T-100 buffer (i.e. 100 mM NaCl). The flow cell was stably mounted to the TIRF microscope using adhesive tape to prevent movement once imaging started. On one of the flow-through inlets of the flow cell, a 1 mL syringe equipped with a length of 28 gauge PTFE rubber tubing (Hamilton) was attached. On the other side of the flow-cell, the flow-through inlet had a reservoir made from an Eppendorf tube cap, with a hole in it, which was attached with epoxy and filled with T-100 buffer containing 61 pM gfpFis. Flow cells are initially imaged in protein-free buffer for a few frames before protein is flowed in using the syringe during imaging. In contrast to the off-rate measurements, the same region of the flow-cell is imaged throughout the experiment to speed up the time resolution. The number of signals in each frame is plotted as a function of time (Fig. S4*A*). In separate experiments, the rate of gfpFis bleaching under the same buffer and imaging conditions is measured and used to correct the on-rate measurement (Fig. S4*B*). The bimolecular on-rate constant is given by $\gamma = (\tau_{\mathrm{on}} c)^{-1}$, where ${\tau_{\mathrm{on}}}^{-1}$ is the on-rate and c is the gfpFis concentration. We checked that ${\tau_{\mathrm{on}}}^{-1}$ had the expected linear scaling with protein concentration by measuring ${\tau_{\mathrm{on}}}^{-1}$ at two different concentrations (Fig. S4*C*). We measure the bimolecular on-rate constant to be $\gamma = 1.04 \pm 0.19 \times 10^8$ $\mathrm{M^{-1}s^{-1}}$. An independent measurement of γ using 243 pM of gfpFis is consistent with the measurement at 61 pM (Fig. S4*D*).

S7. Global fitting of off-rate salt and protein dependence to extended model

Derivation of the fitting function

To obtain the fitting function used to model the off-rate, we absorb the physical parameters $\vec{p}_{\mathrm{phys}}$ that appear in the microscopic kinetic rates k_{ij} into a smaller set of eight fitting parameters $\vec{p}$. With the exception of k_{12}, which is given by γc, this results in the following form for the microscopic kinetic rates

$$k_{\mathrm{ij}} = \alpha_{\mathrm{ij}} {c_{\mathrm{s}}}^{N} \qquad \text{[S18]}$$

where $\alpha_{\mathrm{ij}} = \nu_{\mathrm{ij}} \exp(-\Delta E^{\mathrm{ij}}_{\mathrm{barrier}}/k_{\mathrm{B}}T) \left(\frac{1}{K_{\mathrm{s}}}\right)^{N}$ and $N = n_{01}$, n_{13}, $n_{01} + n_{13}$, or 0 depending on the ij combination given above. We simply refer to α_{10} as k_{10} since it has no salt dependence. Plugging in these forms of k_{ij} into Eq. S10, we obtain

$$k_{\mathrm{off}}(c, c_{\mathrm{s}}; \vec{p}) = \alpha_{01} {c_{\mathrm{s}}}^{n_{01}} [\gamma c\, \alpha_{23} {c_{\mathrm{s}}}^{n_{01}+n_{13}} + \alpha_{13} {c_{\mathrm{s}}}^{n_{13}} (\alpha_{21} {c_{\mathrm{s}}}^{n_{01}} + \alpha_{23} {c_{\mathrm{s}}}^{n_{01}+n_{13}})] / [k_{10} (\alpha_{21} {c_{\mathrm{s}}}^{n_{01}} + \alpha_{23} {c_{\mathrm{s}}}^{n_{01}+n_{13}}) + \alpha_{01} {c_{\mathrm{s}}}^{n_{01}} (\gamma c + \alpha_{21} {c_{\mathrm{s}}}^{n_{01}} + \alpha_{23} {c_{\mathrm{s}}}^{n_{01}+n_{13}}) + \gamma c\, \alpha_{23} {c_{\mathrm{s}}}^{n_{01}+n_{13}} + \alpha_{13}\alpha_{21} {c_{\mathrm{s}}}^{n_{01}+n_{13}} + \alpha_{13}\alpha_{23} {c_{\mathrm{s}}}^{n_{13}} {c_{\mathrm{s}}}^{n_{01}+n_{13}}]. \qquad \text{[S19]}$$

We form a χ^2 statistic to be minimized

$$\chi^2(\vec{p}) = \sum_{i=1}^{N_{\mathrm{m}}} \frac{\left(k_{\mathrm{off},i} - k_{\mathrm{off}}(c_i, c_{\mathrm{s},i}; \vec{p})\right)^2}{{\sigma_{k,i}}^2} \qquad \text{[S20]}$$

where $k_{\mathrm{off},i}$ are the off-rate measurements, ${\sigma_{k,i}}^2$ are the variances in each measurement (Eq. S5), N_{m} is the number of off-rate measurements, and $k_{\mathrm{off}}(c_i, c_{\mathrm{s},i}; \vec{p})$ is the model evaluated at each salt and wtFis concentration measured. We minimize $\chi^2(\vec{p})$ by the simplex method using the *fminsearch*() function in MATLAB (MathWorks). The minimization using the full parameter set was impossible due to the large size of the parameter space. However, we were able to reduce the number of fitting parameters since we fix γ directly by experiment, and, since we found that $k_{23}/k_{21} \gg 1$ for salt concentrations greater than 20 mM (at c_{s} =100 mM k_{23}/k_{21} must be at least 600 and by all indications almost certainly much larger, i.e. up to $\sim 10^8 - 10^9$), we found we could safely neglect k_{21} in comparison to k_{23}. As a result, we are left with 6 fitting parameters: $\vec{p} = (\alpha_{01}, \alpha_{23}, \alpha_{13}, k_{10}, n_{01}, n_{13})$. After some algebra, our fitting function becomes

$$k_{\mathrm{off}}(c, c_{\mathrm{s}}; \vec{p}) \approx \frac{\alpha_{01} {c_{\mathrm{s}}}^{n_{01}} (\gamma c + \alpha_{13} {c_{\mathrm{s}}}^{n_{13}})}{k_{10} + \alpha_{01} {c_{\mathrm{s}}}^{n_{01}} \left(\frac{\gamma c}{\alpha_{23} {c_{\mathrm{s}}}^{n_{01}+n_{13}}} + 1\right) + \gamma c + \alpha_{13} {c_{\mathrm{s}}}^{n_{13}}}. \qquad \text{[S21]}$$

In addition, we can impose constraints on n_{01} and the combination $n_{01} + n_{13}$. First, $n_{01} + n_{13}$ is the power-law exponent of the salt dependence of the spontaneous dissociation pathway, which was measured in Fig. 3*C*, and therefore is to be identified with $M = n_{01} + n_{13} = 2.6 \pm 0.3$. Second, we found that we could identify the power-law exponent, $m < \sim 0.25$, of the weak salt dependence measured at 243 nM wtFis with n_{01} (Fig. 3*C*). This is because the salt-dependence in this regime is

$\approx k_{\mathrm{exch}}c = \frac{k_{01}\gamma c}{k_{10}+k_{01}+k_{13}} \sim {c_{\mathrm{s}}}^{n_{01}}$ (note, this is true since $k_{10} \gg$ both k_{13} and k_{01} in the denominator of $k_{\mathrm{exch}}c$).

Monte Carlo fitting procedure

In Fig. 4*B* (*Main Text*) we perform a global fit of our data with Eq. S21 and estimate confidence intervals of the fit using a Monte Carlo fitting method described below. The fit imposes priors on γ, n_{01}, and $n_{01}+n_{13}$, draws parameter errors from their joint distribution (which is estimated by calculating the covariance matrix (49)), and tests sensitivity to the parameter initial starting values for the simplex method. On each of 60,000 total Monte Carlo runs we:

1. Choose a γ value from a Gaussian distribution centered at the measured value with a standard deviation equal to the measurement uncertainty.
2. Choose an $M = n_{01} + n_{13}$ value from a Gaussian distribution centered at the measured value with a standard deviation equal to the measurement uncertainty.
3. Choose an n_{01} value from an exponential distribution $\left(1/\sigma_{n_{01}}\right)\exp(-n_{01}/\sigma_{n_{01}})$ with $\sigma_{n_{01}} = N_{\mathrm{max}}/4$ and setting $N_{\mathrm{max}} = 0.25$ (the maximum allowable value for the weak salt dependence at 243 nM wtFis). This choice of $\sigma_{n_{01}}$ gives a small probability density beyond $N_{\mathrm{max}} = 0.25$. We chose an exponential distribution to model the uncertainty in n_{01} since the most probable value is $n_{01} = 0$ and we found that this was the best fit value (Fig. 3*C*, *Main Text*). n_{13} is then set by taking the difference between the sampled values (i.e. $n_{13} = M - n_{01}$).
4. Perform a nonlinear minimization of $\chi^2\left(\vec{p}_{\mathrm{optim}}; \gamma, n_{01}, n_{13}\right)$ using the fitting function in Eq. S21 with γ, n_{01}, and n_{13} fixed to their sampled values, and where the parameters being optimized are $\vec{p}_{\mathrm{optim}} = (\alpha_{01}, \alpha_{23}, \alpha_{13}, k_{10})$. Before the nonlinear optimization is performed, the initial starting values $\vec{p}_{\mathrm{optim,0}}$ for the minimization algorithm are randomly varied by 15% (and drawn from a uniform distribution). The best fit values are given by $\vec{p}_{\mathrm{best}} = \arg\min_{\vec{p}_{\mathrm{optim}}} \chi^2\left(\vec{p}_{\mathrm{optim}}; \gamma, n_{01}, n_{13}\right)$.
5. Calculate the covariance matrix Σ of the fit. Σ is given by $(\mathrm{F}^T\mathrm{F})^{-1}s^2$, where the matrix F is given by $F_{ij} = \frac{\partial k_{\mathrm{off}}\left(c_i, c_{\mathrm{s},i}; \vec{p}\right)}{\sigma_{k,i}\, \partial p_{\mathrm{optim},j}}$ where the index i runs over the measurements and the index j runs over the parameters being optimized, and where $s^2 = \frac{{\chi_{\mathrm{min}}}^2}{N_{\mathrm{m}} - N_{\mathrm{p}}}$. $N_{\mathrm{m}} = 19$ is the number of data points and $N_{\mathrm{p}} = 4$ is the number of parameters being optimized. Nearly all the variance of the fit is captured by k_{01} and k_{10}, so in practice we only let j run over those parameters when forming the matrix F.
6. Randomly draw errors in the parameter vectors $\delta\vec{p}_{\mathrm{optim}}$ along the principal axes of their joint distribution, which is assumed to be approximately Gaussian near ${\chi_{\mathrm{min}}}^2$. The principal axes are given by the eigenvectors of Σ, and the variances along those directions are given by the corresponding eigenvalues of Σ (49).
7. Generate off rate curves $k_{\mathrm{off}}\left(c, c_{\mathrm{s}} = 100\mathrm{mM}; \vec{p}_{\mathrm{optim}} + \delta\vec{p}_{\mathrm{optim}}\right)$, $k_{\mathrm{off}}\left(c = 0, c_{\mathrm{s}}; \vec{p}_{\mathrm{optim}} + \delta\vec{p}_{\mathrm{optim}}\right)$, and $k_{\mathrm{off}}\left(c = 243\mathrm{nM}, c_{\mathrm{s}}; \vec{p}_{\mathrm{optim}} + \delta\vec{p}_{\mathrm{optim}}\right)$ evaluated at $\vec{p}_{\mathrm{optim}} + \delta\vec{p}_{\mathrm{optim}}$.

The 1-σ confidence intervals in Fig. 4*B* contain 68.3% of the generated k_{off} curves, and the main curves correspond to the best fit parameters. The fitted values and 68% confidence intervals of the microscopic rate constants are reported in Table 1.

S8. Coarse-grained simulations of facilitated dissociation

Details of simulations including explicitly-modeled salt ions

The simulation model is designed to mimic the single-binding/unbinding experiments with Fis-DNA. Each simulation box contains at least n_{o}=10x10 surface-grafted DNA chains (binding sites), an equal number of protein molecules initially bound onto the grafted DNA chains, a prescribed number of initially unbound proteins, corresponding counterions for protein and DNA molecules to keep the overall charge of the system neutral, monovalent salt ions and an inert surface (see Fig. S5). The aqueous medium is modeled implicitly as a continuum (see below). The DNA and proteins are modeled by a coarse-grained "Kremer-Grest (KG)" bead-spring chain (50). In the model, each bead with size σ represents an interaction site. In real units, σ corresponds to 7Å. The DNA chains are composed of N = 12 adjacently connected, identically sized beads. Four of the beads, which form the binding site, are given attractive interactions. Proteins are composed of p=4 connected identical beads. The bonding between the adjacent beads of the chain is taken care of by a non-linear potential with finite extensibility [ref. K. Kremer, J. Chem. Phys., 1990](50). Steric interactions between all connected and unconnected beads are accounted for by a shifted 12-6 Lennard Jones (LJ) potential with a strength of 1 k_BT and a cut-off distance of $r_{\mathrm{c}} = 2^{1/6}\,\sigma$, where k_B is the Boltzmann constant and T is the temperature. This cut-off is used to obtain a repulsive LJ force between all monomers except the four beads on each DNA chain which form the binding site. Four of the N=12 beads (blue beads in Fig. S5) interact with the proteins via an attractive LJ potential to mimic the specific binding sites for Fis proteins in the experiments. This LJ potential has a strength of $2k_BT$ with a cut-off of $r_{\mathrm{c}} = 2.5\,\sigma$. To account for the stiff nature of the grafted DNA chains, a harmonic bending potential, $U = K\,(\Theta - \pi/2)^2$, is introduced where Θ is the angle formed by three adjacent beads, and the potential strength is given by $K = 30\,k_BT/\mathrm{rad}^2$. In addition, to keep the grafted chains at a right angle with the surface, a similar potential is applied on the grafted end of the chains ($U = 3K\,(\Theta - \pi/2)^2$). The distance between the grafted binding sites is $s = 24\sigma$.

Each effective DNA monomer is assigned a unit negative charge, whereas each protein monomer bears a positive unit charge. For each charged bead of the DNA or protein, one oppositely charged counterion bead is added in the simulation box at a random position so that the system is electro-neutral even in the absence of salt. The monovalent salt (NaCl) concentration is adjusted by adding a prescribed number of positive and negative charged beads in the simulation boxes. The size of the counterion and salt beads is 0.4σ, and they interact with each other via a shifted 9-6 LJ potential. The short range electrostatic interactions between all charged beads are accounted for by imposing a pairwise Coulomb potential $U_{\mathrm{Coul}}(r_{\mathrm{ij}}) = I_{\mathrm{B}}/r_{\mathrm{ij}}$, where r_{ij} is the distance between the ith and jth monomers. The potential is cutoff at $r_{\mathrm{ij}} = 12\sigma$. Note that this cutoff is half the distance between two grafted binding sites on the surface. The long-range electrostatic interactions are calculated via a Particle-Particle-Particle Mesh (PPPM) Ewald solver with an error tolerance of 10^{-3} (51). The

dielectric constant is set to unity so that the Bjerrum length l_B is equal to its value in aqueous medium at room temperature (i.e. $l_B \approx 1\sigma \approx 7$Å) (52, 53). The Bjerrum length defines the length scale, at which electrostatic energy is of the order of the thermal energy ($\sim 1\ k_BT$). Note that the lowest salt concentration (10 mM) considered in this work corresponds to a Debye screening length of $\kappa^{-1} \approx 4\sigma \approx 30$Å.

The MD simulations are run at constant volume V and constant reduced temperature $T = 1.2$ with Lammps MD package (54). The volume of the total simulation box is set to at least 230x230x60 σ^3. The vertical size of the boxes (60 σ) is higher than the effective Gouy-Chapman length of the DNA grafted surface (*i.e.,* $\lambda_{GC} \approx \frac{s^2}{N\ l_B} \approx 30\sigma$). Higher box heights were also tested, but no significant change was observed. The simulations are run with a timestep of $\Delta t = 0.005\tau$, where τ represents the LJ time unit. The temperature is kept constant by a Langevin thermostat with a thermostat coefficient $\gamma = 0.1\tau^{-1}$. Each system is simulated 10^6 or more MD steps at the given temperature. No equation of motion is solved for the surface.

To take into account the unlabeled Fis proteins, the protein chains are added randomly in the simulations box (black chains in Fig. S5). The volume fraction of the proteins is varied between 0 and 10^{-4} s^{-3}. For instance, adding one competing protein per binding site (e.g., 100 competing proteins for 100 sites) corresponds to a volume fraction of $2x10^{-5}$ s^{-3}. Note that these concentrations are higher than those in the experiments. This is purely out of computational necessity and is a reflection of the fact that, in the simulations, the overall binding energy is much weaker than in the experiments thus requiring a larger protein concentration to see an effect. However, the effects of the salt and competing proteins on the un/binding kinetics are captured successfully in the simulations.

Quantification of off-rates

Upon initiation of the data production simulations, the number of proteins remaining bound to the DNAs, $n(t)$, is monitored as a function of time t (Fig. S6). If any bound polymer diffuses out of the spherical volume with radius $R_c \approx 4\sigma$, centered around the binding site, the protein gets tagged as unbound. If a protein returns to the binding site, it doesn't get tagged as bound. To determine the off-rate k_{off} both in the absence and presence of free proteins, the survival fraction data is fit by a single exponential $n(t) = n_o \exp(-k_{off}\ t)$ (Fig. S6). Unless noted otherwise, all simulation results are averaged over time. Error bars are not shown if they are smaller than the size of the corresponding data point. VMD is used for visualizations (55).

SUPPORTING FIGURES

Figure S1. F1 sequences are binding sites for single gfpFis dimers. (A) Sample single molecule fluorescence trajectories for gfpFis signals displaying one (left), two (middle), or three (right) bleach steps. Horizontal red lines represent gfpFis brightness at each fluorescence level. (B) Histogram of the number of bleach-steps observed from each of a total of 50 observed gfpFis trajectories. Error bars are the square root of the number of trajectories in each bin. (C) Histogram of measured bleach-step sizes from a total of 30 Cy3-F1 DNA signal trajectories. The total number of bleach steps is 33 (a minority of Cy3 signals had multiple bleach steps). The mean of the distribution is $\langle B_{\mathrm{sm}} \rangle = 141700 \pm 9800$ counts/signal/350 ms. (D) Overall probability $P(n|n>0)$ to observe n binding sites in a diffraction limited region, given there is at least one binding site. $P(n|n>0)$ combines the probability for a streptavidin molecule to be occupied by σ binding sites with the probability for n_s streptavidin molecules to be colocalized to a diffraction limited area.

Figure S2. Simple model of facilitated dissociation. (A) Kinetic diagram of facilitated dissociation. In a simple version of the kinetic scheme depicted in Eq. S6 (27), the protein molecules and DNA binding sites are each represented by a dimer of identical subunits. Due to the two-fold symmetry implicit in this scenario, the TF partially unbinds by breaking exactly half of the total number of contacts made with the DNA. Note: we do not intend this figure to depict the actual structure of the Fis-DNA-Fis ternary complex nor to suggest that one Fis subunit actually completely dissociates from DNA in state 1. (B) Red curves show the predicted salt-dependence of the off-rate in the facilitated dissociation kinetic scheme using arbitrary parameters. Red curves are parameterized by different protein concentrations. Solid black curve represents the salt-dependence of the spontaneous dissociation pathway, which does not depend on the concentration of TFs in solution, and shows the asymptotic behavior at high salt concentrations. Dashed black curves are parameterized by the protein concentration, and show the asymptotic behavior of the off-rate at low salt concentrations where the protein dependent pathway is dominant.

Figure S3. Energy diagram of FD model including multivalency of TF-DNA interactions and salt ions. Upper diagram corresponds to the spontaneous dissociation pathway. Lower diagram corresponds to the protein concentration dependent pathway. TFs (green and black strings of beads) are composed of multiple identical subunits, each of which binds a part of the DNA binding site (blue strings of beads). Salt ions (red crosses) are explicitly included and are allowed to compete with TFs for making contacts with the DNA binding site. The overall binding energy of a TF is given by E_b. Transition barrier heights are labeled with either *B* (to indicate part or all of a TF is *binding* DNA) or *UB* (to indicate part or all of a transcription factor is *unbinding* DNA). Black arrows represent microscopic kinetic rates between states. Grey arrows are auxiliary rates used in calculating the salt-dependence of the kinetic rates k_{ij} that go in the off-rate calculation.

Figure S4. Measurement of on-rate constant. (A) On-rate measurement uncorrected for bleaching. Red marker designates when gfpFis is added into solution. The concentration of gfpFis added in solution is 61 ± 1 pM. (B) On-rate measurement corrected for bleaching. Data is fit to an exponential recovery function (red curve) of the form $a + A\left(1 - \exp\left(\frac{-t-t_{\mathrm{o}}}{\tau_{\mathrm{on}}}\right)\right)$, with a fixed to 26.25 signals,

giving an exponential recovery time of $\tau_{\mathrm{on}} = 151 \pm 12\ s$. (C) On-rate ${\tau_{\mathrm{on}}}^{-1}$ shows expected linear scaling with gfpFis concentration c. Data points are on-rate measurements at 61 ± 1 pM and 243 ± 5 pM. Error bars represent statistical error in on-rate from fitting to an exponential recovery. Solid line has a slope equal to the measured bimolecular on-rate constant γ estimated from the 61 pM measurement. Dashed lines represent the total statistical error in γ. Systematic error in the on-rate, due to error in the bleaching rate, is represented by duplicate data points at each concentration. (D) Bimolecular on-rate constant γ estimated from 243 pM data is consistent with estimate from 61 pM data. Data points at each concentration are weighted averages of data points in (C). Error bars include all statistical and systematic errors combined. Red line represents a typical estimate of a diffusion limited bimolecular on-rate constant (56).

Figure S5. Illustration of single binding-site model used in the simulations. A small portion of the 3D simulation box is shown on the right. d is the average distance between competitor protein in solution, and s is the distance between two grafted chains. In the simulations, the full number of binding sites is 100.

Figure S6. Survival fraction curves in simulations. (A) Survival fraction of bound proteins as a function of simulation time for various salt concentrations in the presence of competitor Fis. The volume fraction of competitors is on the order of $10^{-5}\sigma^{-3}$. (B) Survival fraction for various competitor protein concentrations at $c_{\mathrm{s}} = 34 \times 10^{-3}\sigma^{-3}$. The simulation time is in the units of LJ time. $n_{\mathrm{o}} = 100$. All curves are single exponential fits.

Figure S7. Heterotypic FD of NHP6A. Survival fraction curve (red) of NHP6Agfp using wtFis as competitor showing that wtFis is able to cause FD of NHP6A. A survival fraction curve of NHP6Agfp with no competitor is reproduced from Fig. 2*C* for comparison.