**Supplement to:**
**The human mitochondrial single strand DNA binding protein displays distinct kinetics and thermodynamics of DNA binding and exchange**

by

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***Global data fitting.***

The kinetic parameters reported in the main text were derived by globally fitting the data in Figure 4 to define the kinetics for dT30 binding, and the data in Figures 5 and 6 to define the kinetics of dT60 binding. In this supplement, we described the setup of KinTek Explorer to fit the kinetic and equilibrium binding data simultaneously and show the results of confidence contour analysis to establish that the kinetic parameters were well constrained by the data.

***Kinetics of dT30 binding.*** We first show the fitting of data shown in Figure 4 of the main text.

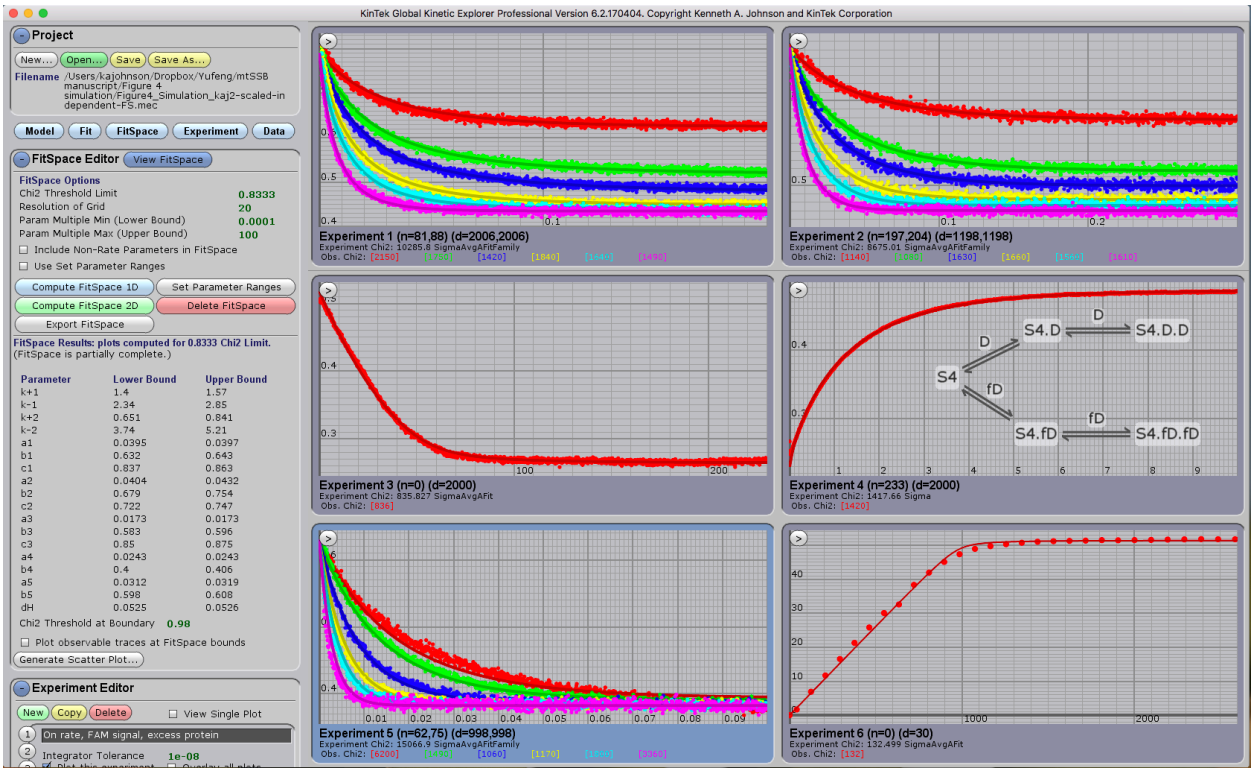



**Figure S1**. *Kinetics of dT30 binding to mtSSB*. This figure shows a Screen capture of KinTek Explorer software showing the fitting of 6 experiments simultaneously for data shown in Figure 4. The inset model shows mtSSB (S4) binding to either DNA (D) or fluorescently labeled DNA (fD).

The model was set up with identical reactions for the binding of unlabeled and fluorescently labeled DNA. Here we use S4 to indicate the mtSSB tetramer, capable of binding two dT30 molecules of ssDNA. D designates DNA and *f*D designates fluorescently labeled DNA. To maintain equality for the rate constants for labeled and unlabeled DNA, we use the linked group function in KinTek Explorer as shown in Figure S2.

$$S4 + DNA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S4.DNA \qquad S4.DNA + DNA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} S4.DNA.DNA$$

**Figure S2**. *Modeling the two–step reaction for labeled and unlabeled DNA*. The reactions are listed explicitly as shown on the right and then the rate constants are grouped to ensure identical rate constants for labeled and unlabeled DNA : group *a* for $k_1$, group *b* for $k_{-1}$, group *c* for $k_2$, and group *d* for $k_{-2}$.

| Reactions | k+ | k- |
|---|---|---|
| S4 + D = S4.D | (a) 1.48 | (b) 2.59 |
| S4.D + D = S4.D.D | (c) 0.748 | (d) 4.39 |
| S4 + fD = S4.fD | (a) 1.48 | (b) 2.59 |
| S4.fD + fD = S4.fD.fD | (c) 0.748 | (d) 4.39 |

Each experiment is defined by the starting concentrations for reactants and the output signal, which is encoded by a mathematical relationship involving the concentrations of individual species and output scaling factors (a, b, c as define below) that relate concentrations to the observable signal. Individual Experiments to define dT30 binding kinetics (main text Figure 4) are shown in Figure S1. The setup for each experiment is detailed below:

Experiment 1 (Figure 4A). *DNA binding kinetics monitoring protein fluorescence*. Reaction of 20nM mtSSB with 15, 30, 40, 60, 80, and 120 nM dT30 DNA (final concentrations). The signal was modeled as: a1*(S4+b1*(S4.D + c1*S4.D.D)), where S4 represents mtSSB, D represents dT30 DNA, and a, b and c are fluorescence scaling factors.

Experiment 2. (Figure 4B) *Reaction of second dT30 with a preformed mtSSB.dT30 complex*. Equimolar MTSSB and dT30 (42 nM each) were allowed to equilibrate, then diluted 1:1 when mixing with various concentrations of excess dT30 (14, 30, 40, 60, 80, and 120 nM, final concentrations). The protein fluorescence was monitored to measure the kinetics of binding the second dT40 to each MTSSB. The signal was modeled as: a1*(S4+b1*(S4.D + c1*S4.D.D)).

Experiment 3. (Figure 4C). *Titration of mtSSB with ssDNA, monitoring protein fluorescence.* A solution containing 30 nM mtSSB was titrated with 0-230 nM ssDNA (dT30) while recording protein fluorescence. The signal was modeled as: a3*(S4+b3*(S4.D + c3*S4.D.D))

Experiment 4. (Figure 4E). *DNA dissociation rate monitoring FAM-DNA fluorescence*. MTSSB (40 nM) was allowed to equilibrate in binding 44 nM FAM-DNA, then diluted 1:1 while mixing with an excess (600 nM final concentration) of unlabeled DNA. The FAM fluorescence then defined the rate of dissociation. The signal was modeled as: a4*(D+ b4*(S4.D + c4*S4.D.D))

Experiment 5. (Figure 4D). *DNA binding kinetics with FAM-DNA fluorescence*. Reaction of 20 nM FAM-DNA with 40, 50, 80, 120, 160, and 240 nM mtSSB (final concentrations). The signal was modeled as: a5*(S4+b5*(S4.D + 2*S4.D.D)) so that the amplitude of the signal was equal for the binding of each DNA.

Experiment 6. (Figure 4F). *ITC titration of mtSSB with ssDNA*. 490 nM mtSSB was titrated with 0-2000 nM dT30 while recording changes in heat. The integrated heat signal was modeled as: dH*(S4.D + 2*S4.D.D).

After globally fitting the data, we performed confidence contour analysis to determine the extent to which each kinetic parameter and scaling factor was constrained by the data. All parameters, including the scaling factors were allowed to vary in finding confidence contours for each of the rate constants. This analysis shows that all 4 rate constants (unlinked) and 13 scaling factors were well constrained by the data to demonstrate that the model was not overly complex.

The standard error threshold in $\chi^2$ was calculated from the F-distribution, where $n$ = number of data points and $p$ = number of parameters. We plot the reciprocal of the normalized $\chi^2$ by the $\text{ratio} = \chi^2_{\min}/\chi^2$ to more clearly show the range of values and the best fit.

$$\chi^2_{threshold} = \chi^2_{\min} \cdot \left(1 + \frac{p}{n-p} F^{\alpha}_{p,n-p}\right)$$

$F^{\alpha}_{p,n-p}$ = the F distribution for 1-$\alpha$ error limits

$$\text{ratio threshold} = \chi^2_{\min}/\chi^2_{threshold}$$

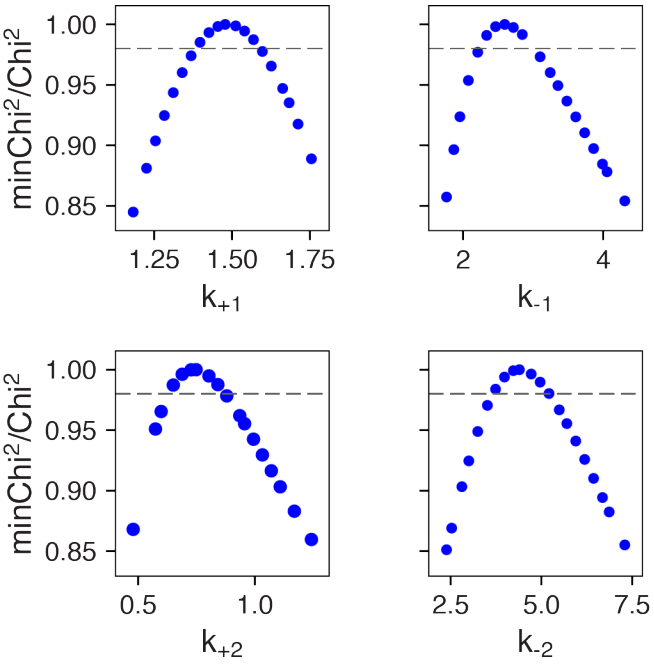



**Figure S3**. *Confidence contour analysis for rate constants*. Each panel shows the reciprocal of the normalized $\chi^2$ (Chi²) value obtained in re-fitting the data while holding one rate constant at a time at the fixed valued given in the x-axis. The dashed line shows the threshold at 0.98 defining lower and upper limits for each rate constant, which are summarized in Table S1, along with the lower and upper limits for the scaling factors derived from the same analysis. The standard error threshold, based on the F-distribution and the number of variables and data points, was computed to be 0.999. Our use of 0.98 provides a more conservative assessment of the confidence intervals. These results demonstrate that the parameters are well constrained by the data.

**Table S1.** *Kinetic parameters derived confidence contour analysis.*

| parameter | best-fit | lower | upper |
|---|---|---|---|
| $k_{+1}$ | 1.48 | 1.4 | 1.57 |
| $k_{-1}$ | 2.59 | 2.34 | 2.85 |
| $k_{+2}$ | 0.748 | 0.651 | 0.841 |
| $k_{-2}$ | 4.39 | 3.74 | 5.21 |
| a1 | 0.0396 | 0.0395 | 0.0397 |
| b1 | 0.638 | 0.632 | 0.643 |
| c1 | 0.849 | 0.837 | 0.863 |
| a2 | 0.0416 | 0.0404 | 0.0432 |
| b2 | 0.72 | 0.679 | 0.754 |
| c2 | 0.733 | 0.722 | 0.747 |
| a3 | 0.0173 | 0.0173 | 0.0173 |
| b3 | 0.589 | 0.583 | 0.596 |
| c3 | 0.863 | 0.85 | 0.875 |
| a4 | 0.0243 | 0.0243 | 0.0243 |
| b4 | 0.403 | 0.4 | 0.406 |
| a5 | 0.0315 | 0.0312 | 0.0319 |
| b5 | 0.603 | 0.598 | 0.608 |
| dH | 0.0526 | 0.0525 | 0.0526 |

The best-fit parameter values are shown with upper and lower limits obtained from confidence contour analysis. Rate constants $k_{+1}$ and $k_{+2}$ have units of $nM^{-1}s^{-1}$, while $k_{-1}$ and $k_{-2}$ have units of $s^{-1}$. The scaling factors **a** have units of volts/nM while the scaling factors **b** and **c** are dimensionless numbers showing the fractional change in fluorescence. The parameter dH is the heat released per unit concentration (nM) in the experiment.

***Kinetics of dT60 binding.*** We now show the fitting of the data shown in Figures 5 and 6.

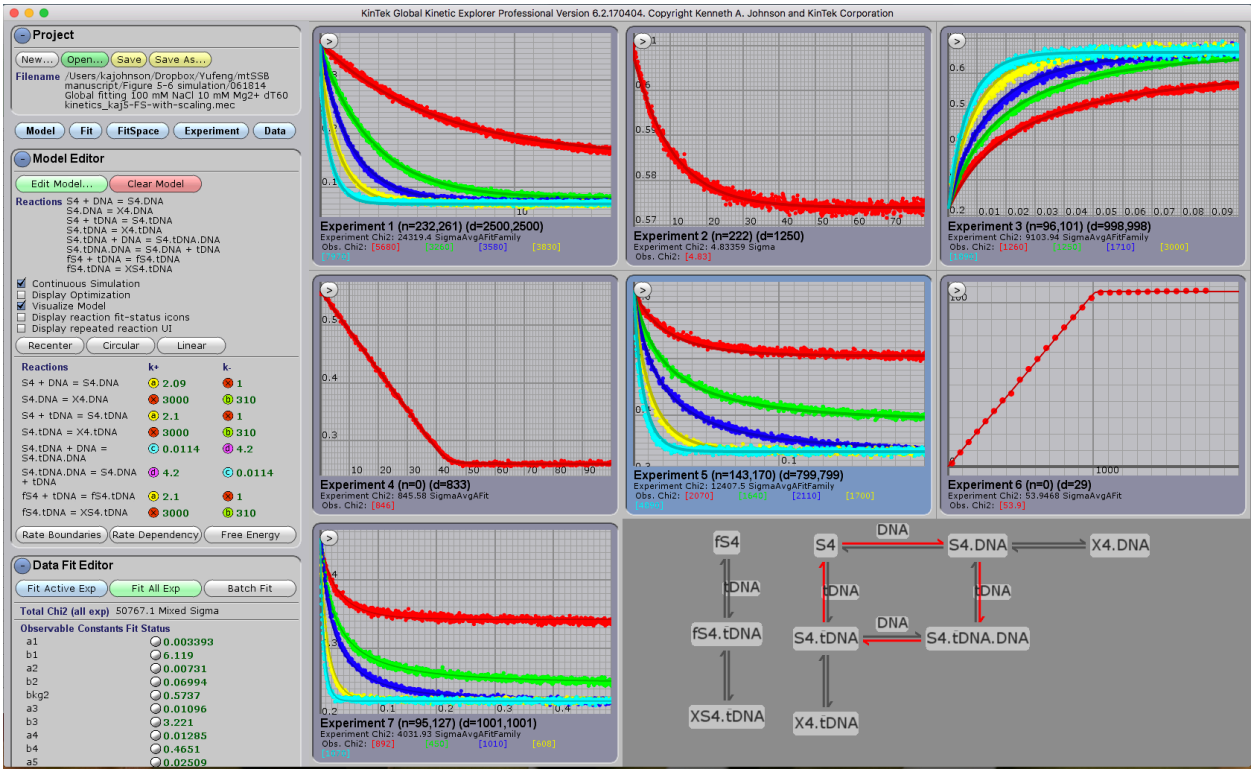



**Figure S4.** *Kinetics of MTSSB binding to dT60.* This figure shows a screen capture of the KinTek Explorer software for the global fitting of data shown in Figures 5 and 6.

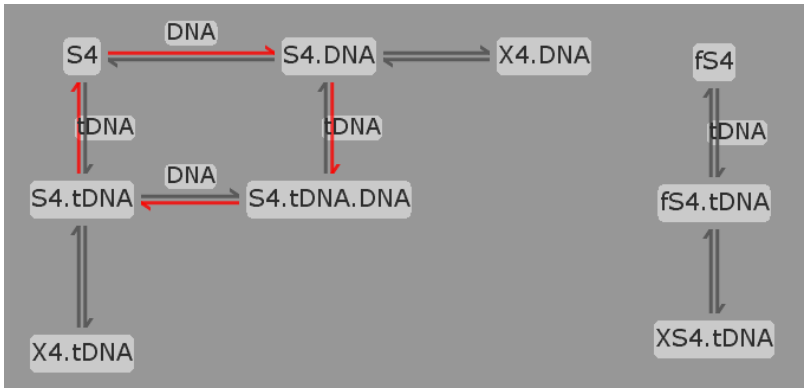
The basic model for MTSSB binding to dT60 is given as:

$$S4 + DNA \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S4.DNA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} X4.DNA$$

were S4 represents the MTSSB tetramer, S4.DNA represents the unwrapped initial complex and X4.DNA represents the *wrapped* MTSSB.DNA complex.

The model was repeated for the identical reactions with DNA labeled with Cy3 and Cy5 to provide a FRET signal with the labeled DNA designated as tDNA, and fluorescently labeled mtSSB, designated as *f*S4. Including the formation on an exchange intermediate, S4.tDNA.DNA leads to the complete pathway shown below.

**Figure S5**. *Model layout.* This figure shows the screen capture of the mechanism display. Note that the closed loop designated by the red lines defines a thermodynamic loop. In the process of data fitting, the product of equilibrium constants going around the loop was maintained at unity.



The individual reaction steps are entered as shown below with rate constants grouped to maintain identify of rate constants for identical reactions for labeled and unlabeled species.

| Reactions | k+ | k- |
|---|---|---|
| S4 + DNA = S4.DNA | (a) 2.1 | (x) 1 |
| S4.DNA = X4.DNA | (x) 3000 | (b) 310 |
| S4 + tDNA = S4.tDNA | (a) 2.1 | (x) 1 |
| S4.tDNA = X4.tDNA | (x) 3000 | (b) 310 |
| S4.tDNA + DNA = S4.tDNA.DNA | (c) 0.0114 | (d) 4.2 |
| S4.tDNA.DNA = S4.DNA + tDNA | (d) 4.2 | (c) 0.0114 |
| fS4 + tDNA = fS4.tDNA | (a) 2.1 | (x) 1 |
| fS4.tDNA = XS4.tDNA | (x) 3000 | (b) 310 |

| Rate constant | Group or value |
|---|---|
| $k_1$ | *a* |
| $k_{-1}$ | (1) |
| $k_2$ | (3000) |
| $k_{-2}$ | *b* |
| $k_3$ | *c* |
| $k_{-3}$ | *d* |

**Figure S6**. *Rate constant list.* This figure shows the screen capture listing rate constants and showing the groups used to maintain a 1:1 ratio of each of the rate constant that is duplicated for the labeled DNA or mtSSB (S4). Constants with the red x are locked at the value listed. Rate constants are defined as in Scheme 2.

Seven experiments were fit simultaneously to derive estimates for the four rate constants. Each experiment is defined by the starting concentrations of reactants and the output signal, defined mathematically to relate concentrations of species to the observable signals.

Experiment 1. (Figure 6A) *DNA exchange rate, FRET signal*. mtSSB (40 nM) and tDNA (40nM) were allowed to equilibrate, then were diluted 1:1 during the addition of unlabeled DNA at concentrations of 50, 500, 1000, 2000, and 4000 nM (final concentrations). The fluorescence signal was modeled as: a1*(tDNA+S4.tDNA+S4.tDNA.DNA +b1*(X4.tDNA)).

Experiment 2. (Figure 5D) *DNA dissociation rate, labeled mtSSB*. Fluorescently labeled mtSSB (62 nM) was allowed to equilibrate with tDNA (60 nM), then diluted 1:1 with the addition of 5000 nM unlabeled MTSSB. The fluorescence signal was modeled as: a2*(tDNA+*f*S4.tDNA +b2*(XS4.tDNA)) +c2.

Experiment 3. (Figure 5C) *DNA binding and wrapping rate using FRET signal*. Fluorescently labeled tDNA (20 nM) was mixed with 20, 30, 40, 60, and 80 nM MTSSB (final). The FRET signal was modeled as: a3*(tDNA+S4.tDNA+b3*(X4.tDNA)) to report a change in fluorescence with DNA wrapping.

Experiment 4. (Figure 5E) *Protein fluorescence titration*. MTSSB (44 nM) was titrated with 0-100 nM DNA. The output signal was modeled as: a4*(S4+b4*(S4.DNA+X4.DNA)) to report a change in protein fluorescence upon DNA binding.

Experiment 5. (Figure 5A) *DNA binding rate, protein fluorescence*. MTSSB (25 nM) was mixed with 10, 20, 30, 50, 80 nM DNA and protein fluorescence was recorded versus time. The signal was modeled as: a5*(S4+b5*(S4.DNA+X4.DNA)). Note that the same fluorescence state was used for both S4.DNA and X4.DNA; although one might expect a difference, the data do not allow a distinction apparently because S4.DNA does not accumulate to significant levels.

Experiment 6. (Figure 5F) *ITC titration of MTSSB with dT60*. MTSSB (1010 nM) was titrated with 0-2000 nM DNA dT60 while recording the heat change. The data from the instrument was integrated (cumulative sum) to get the net heat. The signal was modeled as: ΔH*(S4.DNA+X4.DNA).

Experiment 7. (Figure 5B) *DNA binding rate, FAM-DNA fluorescence*. FAM-labeled DNA dT60 (20 nM) was mixed with 10, 18, 24, 45, 80 nM MTSSB and the change in FAM fluorescence was recorded. The signal was modeled as: a7*(DNA + b7*(S4.DNA + X4.DNA)).

**Figure S7**. *Confidence contour analysis for rate constants*. Each panel shows the reciprocal of the normalized $\chi^2$ (Chi²) value obtained in re-fitting the data while holding one rate constant at a time at the fixed valued given in the x-axis. The dashed line shows the threshold at 0.98 defining lower and upper limits for each rate constant, which are summarized in Table S2, along with the lower and upper limits for the scaling factors derived from the same analysis. The calculated threshold based on the F-distribution and the number of variables and data points was computed to be 0.999. Our use of 0.98 provides a more conservative assessment of the confidence intervals. These results demonstrate that the parameters are well constrained by the data

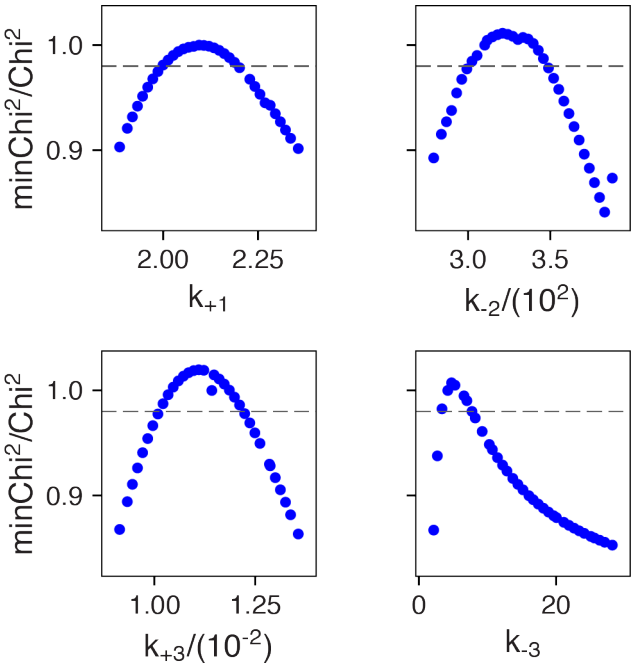



**Table S2.** *Kinetic parameters for dT60 binding derived confidence contour analysis.*

| parameter | best-fit | lower | upper |
|---|---|---|---|
| $k_{+1}$ | 2.09 | 2 | 2.19 |
| $k_{-2}$ | 325 | 302 | 349 |
| $k_{+3}$ | 0.0114 | 0.0102 | 0.0121 |
| $k_{-3}$ | 4.2 | 3.36 | 7.73 |
| *a1* | 0.00339 | 0.00339 | 0.00347 |
| *b1* | 6.12 | 6 | 6.25 |
| *a2* | 0.00731 | 0.00722 | 0.00742 |
| *b2* | 0.0699 | 0.0699 | 0.07 |
| *c2* | 0.574 | 0.574 | 0.574 |
| *a3* | 0.011 | 0.0109 | 0.0112 |
| *b3* | 3.22 | 3.16 | 3.22 |
| *a4* | 0.0129 | 0.0129 | 0.0129 |
| *b4* | 0.465 | 0.465 | 0.465 |
| *a5* | 0.0251 | 0.025 | 0.0252 |
| *b5* | 0.535 | 0.534 | 0.536 |
| *dH* | 0.106 | 0.106 | 0.106 |
| *a7* | 0.023 | 0.0229 | 0.0231 |
| *b7* | 0.483 | 0.482 | 0.485 |

The best-fit parameter values are shown with upper and lower limits obtained from confidence contour analysis.

*Rate of mtSSB binding in the presence and absence of $Mg^{2+}$.* The data in Figure S8 show the DNA concentration dependence of the rate of DNA binding to mtSSB, monitored by the protein fluorescence signal, in the presence and absence of $Mg^{2+}$. The data show that the Mg2+ concentration does not affect the rate constant for DNA binding. The tighter binding seen in the absence of $Mg^{2+}$ is presumably due to a slower dissociation rate.

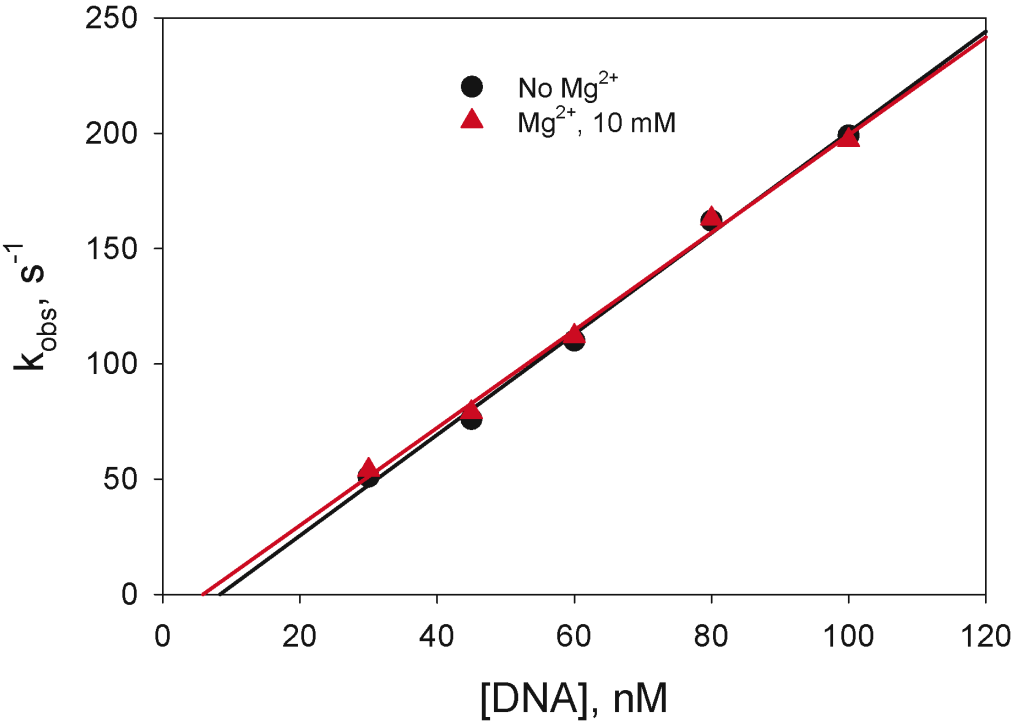



**Figure S8**. *Concentration dependence of the rate of DNA binding to mtSSB in the presence and absence of $Mg^{2+}$.* The rate of DNA binding was measured by stopped-flow fluorescence methods following the change in protein fluorescence of mtSSB after mixing with excess $dT_{60}$ in 100 mM NaCl and 10 mM $MgCl_2$. The data were fit to a single exponential to obtain an estimate of the rate of binding (red triangles). The slope of the plot of the observed rate versus DNA concentration affords a second order rate constant of 2.1 ± 0.1 $nM^{-1}s^{-1}$. A similar experiment was performed to monitor the rate of binding in the absence of $MgCl_2$ (including the 100 mM NaCl, black circles), to obtain an identical second order rate constant.