Supplementary Information

The following items of supporting material are provided as an addendum to “Molecular dynamics of a κB DNA element: Base flipping via cross-strand intercalative stacking in a microsecond-scale simulation” by Mura & McCammon:

- Methods of procedure and simulation details (pgs. S2–4)
- Supplementary figure captions (pgs. S5–8)
- Supplementary references (pg. S9–10)
- Supplementary figures (8)
- Supplementary animations (4)
Methods of Procedure

Computational procedures occurred over roughly three stages, consisting of (i) judicious system selection, construction, and preparation for molecular dynamics (MD) simulations in explicit solvent, (ii) calculation of equilibrium MD trajectories \textit{via} numerical integration of the classical equations of motion, and (iii) data processing and analyses of simulation results.

System selection, construction, & preparation

The canonical binding sites for NF-κB – \textit{i.e.}, the consensus sequences of κB DNA elements – vary among homo- and hetero-dimeric members of the Rel/NF-κB family of transcription factors. The double-stranded (ds) κB DNA structure chosen as the starting point for the studies reported herein is a 20-base pair (bp) duplex drawn from the X-ray crystallographic structure of an NF-κB homodimer (specifically, the (c-Rel)–proto-oncoprotein) bound to a conserved κB DNA regulatory sequence from the CD28 response element located upstream of the \textit{interleukin-2} gene. This structure was determined by Ghosh and co-workers (1) to a resolution of 2.85 Å (PDB accession code 1GJI), and features a nonamer κB DNA recognition element of sequence $^{5'}$AGAATTCC$^{3'}$ (half sites recognized by individual NF-κB monomers are underlined) embedded within the non-palindromic 20-bp sequence $^{5'}$GGTTAAAGAATTCCAG$^{3'}$ (the κB element is underlined). This DNA contains two κB half sites which together form a composite, pseudo-palindromic cognate NF-κB–binding site (see also Fig. S1).

As described by Huang \textit{et al.} (1), the crystals used to determine the structure of the ternary complex of (c-Rel)$_2$•κB DNA were found to exhibit a peculiar form of static disorder, wherein DNA duplexes in different unit cells exist as a 1:1 mixture of two conformations differing in their exact binding orientation to c-Rel; therefore, the 1GJI model consists of two DNA configurations, each having been refined at 50% occupancy. Structural statistics for both DNA models are comparable (\textit{e.g.}, nearly identical mean $B$-factors), and they can be superimposed to a least-squares coordinate RMSD of 2.3 Å (differences occur primarily toward the duplex terminus which is most distal to the κB site); thus, the starting DNA structure was taken as the conformer featuring a slightly greater number of protein–DNA contacts. (Despite its moderate resolution and this 1:1 disorder, we opted to use the 1GJI κB DNA structure rather than to re-build the same κB DNA sequence into an ideal, canonical $B$-form helix, because the structural and dynamical properties of this particular (c-Rel)$_2$•κB DNA complex are of interest as part of a broader investigation.)

Construction of the simulation system (Figs. S1a,b,c) began by positioning the κB DNA element at the center of a suitable truncated octahedral (T.O.) periodic boundary condition (PBC) cell. The diameter of the minimally circumscribing sphere for the dsDNA duplex \textit{i.e.}, the region of space swept-out in the long-time limit of isotropic rotational tumbling; Fig. S1d) was calculated as being $\approx 74$ Å (for a cylindrically symmetric molecule such as DNA, this is roughly twice the distance from the center-of-mass to the most distant atom from this center); in addition, calculations of the five distinct hydrodynamic characteristic times for rotational relaxation of this 3D body (Fig. S1e) \textit{via} the hydrodynamic shell methodology implemented in the HYDROPRO suite (2) indicated that the 20-bp DNA would undergo significant rotational self-diffusion on an $\approx 20$-ns timescale. Together with the goal of simulating the dsDNA duplex for ultra-long times (relative to rotational diffusion relaxation times) without the imposition of restraints, these geometric parameters led to construction of a conservative PBC cell, with a $d_0 = 94.0$ Å distance between the geometric centers of the hexagonal faces of the truncated octahedron (corresponding to $d_4 = 108.5$ Å across the square faces of the geometrically regular T.O.). The dsDNA was centered at the origin of this T.O. cell and re-oriented such that its helical axis was coaxial with the normals between one of the three pairs of square faces of the T.O. (taken as the $z$-axis of the new reference frame). Barring unexpectedly massive deformation (\textit{i.e.}, unfolding) of the DNA along its helical axis during the course of this equilibrium simulation, this PBC cell geometry insures a minimal distance of 20.0 Å between periodic images (this minimal clearance being reached upon diffusional re-orientation of the duplex away from the $d_4$ starting face and towards one of the four shorter ($d_0$) directions).

Upon designing an appropriate PBC geometry as described above, the DNA simulation system was prepared \textit{via} stages of: (i) addition of hydrogens and simultaneous construction of molecular topology, parameter, and associated files for force field-based calculations, using the psfgen module of the VISUAL MOLECULAR DYNAMICS (VMD) program (3) and its associated Tcl and Python application programming interfaces (APIs); (ii) optimization of hydrogen positions and relaxation of the overall structure \textit{via} multiple stages of energy-
minimization (minimizing just the protons, followed by protons and harmonically-restrained DNA atoms, etc.); (iii) placement of 38 Na+ counter-ions at energetically favorable locations about the DNA using the VMD-affiliated ‘SODIUM’ code (4); (iv) placement of an initial hydration shell of interstitial waters around the neutralized DNA•Na+ system (the moderate-resolution 1GJI structure lacked crystallographic water molecules, so 45 waters were placed at energetically favorable positions using a version of the DOWSER software (5) that was customized so as to be able to solvate nucleic acids (6)); and (v) addition of an energetically-favorable environment of bulk water and ions (Na+ and Cl-) to arrive at a large (over-sized) rectangular cell, using the SOLVATE program (7) in conjunction with VMD-based scripts. The corners of this over-sized box were then exactly trimmed to obtain a final truncated octahedral PBC cell matching the aforementioned geometric criteria. Thus, the final 61,439-atom simulation system consisted of a protonated and energy-minimized κB DNA element situated in a T.O. cell (Figs. S1b,c), accompanied by (i) 20,030 molecules of transferable intermolecular potential 3-point (TIP3P) water (8), (ii) 38 Na+ counterions to neutralize the charges of the DNA phosphate groups and ensure electroneutrality (for calculation of lattice sums via the particle mesh Ewald (PME) method), and (iii) an additional 20 Na+ and 20 Cl- ions, providing a final [NaCl] ≈ 50 mM (chosen so as to mimic as closely as possible experimental DNA-binding studies with this and related κB elements (9)). As a final preparation step for MD simulations, the DNA was allowed to adapt to the aqueous ionic environment (and, likewise, the solvent structure to relax around the DNA and remove unfavorable contacts) by minimizing the potential energy of the entire system over a total of 10,000 steps, using the conjugate gradient method implemented in the NAMD simulation code (10); the initially stiff harmonic restraints that were applied to the DNA atoms (e.g., force constants of ≈ 50 kcal/mol/Å2) were successively relaxed to zero for the final 3,000 steps.

**Molecular dynamics simulations**

MD simulations consisted of a heating and equilibration period, followed by 1,020.0-ns of free, production-level dynamics. The 1.0-ns equilibration phase commenced by heating the system in the canonical ensemble (constant NVT), and proceeded via the following multi-stage strategy: (i) heating from 0 → 300 K over a 65-ns period with relatively stiff harmonic restraints on DNA atoms (k ≈ 50 kcal/mol/Å2); (ii) an additional 35-ns of NVT dynamics at 300 K with moderately weakened restraints on DNA atoms (k ≈ 25 kcal/mol/Å2); (iii) continued dynamics in the isothermal-isobaric (NPT) ensemble to permit relaxation and equilibration of the system volume, with restraints on DNA atoms successively relaxed from 25 kcal/mol/Å2 to zero over a 300-ns period; and (iv) a final 600-ns of unrestrained NPT dynamics at 300 K. All production simulations were performed in the NPT ensemble under the truncated octahedral periodic boundary conditions described above, with pressure and temperature maintained at constant values of 1 atm and 300 K, respectively. Temperature was controlled via Langevin dynamics (damping coefficient of 1 ps⁻¹; hydrogen atoms were not coupled to the heat bath), and pressure was regulated by a hybrid Nosé-Hoover Langevin piston (11) (oscillation period of 200 fs and damping decay time of 100 fs). Long-range electrostatic interactions were computed via the smoothed PME algorithm (12), with a minimal grid density of 1/Å in every direction. Non-bonded, short-range (van der Waals) interactions were calculated within a spherical cutoff of 10 Å, with a smooth switching function employed between 9→10 Å in order to avoid abrupt truncation (neighbor lists computed within 11.5 Å). The SHAKE algorithm (13) was utilized to constrain bonds between hydrogens and parent heavy atoms, thereby enabling a 2.0-fs integration time step to be used without deleterious effects on either conservation of bulk thermodynamic quantities (Fig. S2) or overall structural stability over the entire trajectory (Figs. S2,3). Additional computational efficiency was afforded by utilizing a multiple time-stepping scheme (14), with evaluation increments of 2-fs between bonded interactions, 2-fs for short-range non-bonded interactions, and 4-fs for long-ranged electrostatics. (Note that the NAMD simulation code features a smooth splitting function to separate the quickly varying short-range portion of the electrostatic interactions from the more slowly-varying (long-range) components, and the latter 4-fs time step has become routine in simulations using this software.)

Trajectories were computed in 1.0-ns bins via the fully-atomistic, explicit-solvent MD method described above, with coordinates written in high-precision binary format every 1.0 ps. Trajectories were extended to the μs-scale timescale reported herein via a set of scripts that enabled the propagation and linkage of time slices i → i+1 in a semi-automated, minimally-supervised manner, and were computed over extensive periods of wallclock time (nearly one year) on a host of available Linux clusters (all of identical 32-bit architecture); the final collection of trajectories amounts to roughly one terabyte of DNA simulation data. Optimization of the
computational conditions in terms of both software (e.g., a highly-scalable code such as NAMD) and hardware (e.g., multi-core architectures and low-latency, high-bandwidth network fabrics) were found to be indispensable in achieving the simulation timescale reported here.

The calculations described above utilized the NAMD code (15) with the standard AMBER parm94 force field (16). Among the several force fields commonly used in biomolecular simulations, the parm94 version of the transferable second-generation Cornell et al. force field (16) was chosen primarily for the sake of consistency and comparability with the numerous existing DNA simulations that have used this particular parameter set. Compatibility between the closely related parm98/99 AMBER force field and the algorithmic implementations in the NAMD software (e.g., using Langevin dynamics as a means of temperature control) has been demonstrated recently (17), it having been concluded that this approach “sets the stage for reliable large-scale simulation of nucleic acids using NAMD.” Of the available AMBER parameter sets, both methodological and case studies (i) have found parm94 and its parm98/99 variant “to essentially be at the same level of approximation” (18); (ii) concluded that simulations “do not appear to be profoundly sensitive to the choice of drug or DNA force field” (19); and (iii) deemed the AMBER force fields as “roughly equivalent” (20). A similar perspective with regards to the suitability of various implementations of the AMBER force field has been echoed in a recent review of empirical force fields (21). Perhaps most notably, parm94 is the force field of choice in the Ascona B-DNA Consortium’s recent large-scale and systematic simulations of all 136 unique tetranucleotides (22,23). These points constitute an incomplete list which nevertheless serves to highlight the somewhat subjective character of force field selection and application; force field development is an active area of research, with, for example, the parmbsc0 successor to parm98/99 having been very recently released (24). Taken together with the historical record of force fields usage in DNA simulations and the other approximations and simplifications inherent in both force fields and MD methods, the foregoing issues guided our final force field selection.

Data processing & analysis

Trajectory analysis utilized many available tools as well as custom-written scripts, and made particularly heavy use of the Tcl and Python APIs of the VMD (25) and PYMOL (26) molecular graphics/analysis suites. As an example of the issues specifically arising in a simulation of long timescales, self-diffusion of the DNA solute led to undesirable coordinate wrapping behavior about the truncated octahedral faces of the PBC box at long times: in water at 300 K, it takes \( \approx 200 \) ns for the center-of-mass of the 20-bp DNA solute to traverse the \( \approx 30 \text{ Å} \) to the nearest box edge. This problem arose from the independent wrapping of coordinates for two individual DNA strands (naturally treated as two separate, unlinked segments by the simulation software), and necessitated the development of generic “unwrapping” routines capable of handling arbitrarily-shaped boxes (i.e., non-rectangular, oblique cells such as truncated octahedra) in order to properly re-generate coordinates for ‘mis’-wrapped strands. This issue was initially detected as anomalous spikes in long-time “sliding” RMSD values (calculated as the RMSD between frames \( t_n, t_{n-1} \); Fig. S3), was verified by visual inspection of simulation results, and was resolved as part of the trajectory post-processing phase. Structural and conformational analyses of DNA trajectory data in terms of the conventional stereochemical and helicoidal parameters were performed using the CURVES (27) and 3DNA (28) software packages (see, e.g., the sets of parameters plotted in Figs. S6, S7); further data post-processing and analysis utilized programs written in the MATLAB numerical computing environment.
Supplementary Figure Captions

Fig. S1: Overview of the κB DNA simulation system: Sequence, structure, and hydrodynamic properties. A μs-scale MD simulation was performed for a κB DNA element immersed in a bath of explicit water as solvent, with the system set-up under truncated octahedral periodic boundary conditions (a) in order to minimize the number of waters necessary for a given minimum periodic image distance. Perpendicular views of an individual unit cell from the DNA + NaCl system (b, c) show the simulation system consisting of a 20-base pair κB DNA duplex (tan-colored ribbon backbone with bases drawn as stick models), 58 Na⁺ ions (38 counterions added to neutralize the phosphodiester backbone and 20 co-ions), 20 Cl⁻ ions, and roughly 20,000 water molecules; the excess ions were added so as to achieve an [NaCl] ≈ 50 mM. The deoxyribose moieties of the two strands are also drawn as red (strand 1) and blue (strand 2) pentagons, and semi-transparent CPK spheres are shown for bases in the κB recognition element. The counter- and co-ions are drawn as yellow—(Na⁺) and purple—(Cl⁻) colored van der Waals spheres, and the interstitial water molecules closely associated with the DNA (placed in a pre-equilibration step) are indicated as transparent vdW spheres; remaining water molecules are not drawn for the sake of clarity, but the convex envelope defined by this bulk water is rendered as a cut-away rippled surface (blue). Dashed white lines indicate the clipping planes in the two perpendicular views of the truncated octahedron. In addition to providing the sequence of the κB DNA element used in these studies, panel (d) indicates the location of the binding site for the (c-Rel)₂ homodimer (and related members of this NF-κB subfamily). The nomenclature (and nucleotide and base pair numbering scheme) used throughout the text are indicated in this panel, as are the most significant structural/dynamical findings described in the text. The principal directions of rotational self-diffusion of the cylindrically-shaped DNA duplex are indicated by dashed arrows, and the calculated relaxation properties for rotational tumbling of the κB DNA element (e) included a rotational correlation time of ≈ 20 ns for end-over-end tumbling of the duplex; the five unique characteristic times which describe the rotational self-diffusion of a three-dimensional object were calculated for an atomically-detailed model of the κB DNA element using different values of bead element radii, as described in the above Methods section. The two principal relaxation times for a simplified (rigid-rod cylinder) model of DNA are indicated as dashed grey lines (calculated using equations 13-16 of Eimer et al. (29)), and describe rotation about the helical axis (τᵢ) as well as end-over-end tumbling perpendicular to this axis (τ⊥). Because the transverse tumbling time is orders of magnitude less than the target μs timescale of the MD simulation (and because the position of the DNA element was not constrained during the simulation), the principal component of the moment of inertia tensor of the duplex (i.e., the DNA helical axis) was oriented along the longest body diagonal of the truncated octahedron (between square faces), thereby maximizing periodic image-image distances at the start of the simulation, and insuring a minimal image→image distance of 20 Å throughout the course of the simulation (see Methods section).

Fig. S2: Thermodynamics equilibration and stabilization of the κB DNA system. The time-evolution of several thermodynamic variables were monitored to gauge successful equilibration and overall stability of the bulk properties of the system over the entire length of the trajectory. Because of the unprecedented length of this DNA simulation, quantities were monitored over both short (ns; insets) and long (μs) timescales. In each case, data values are shown as points, with window-averaged values plotted as solid (red) traces [calculated using 1-ns (full-length trajectories) or 0.1-ns windows (short-time insets)]. Displayed quantities include total system energy (a), kinetic (b) and potential (c) energies, system temperature (d), bulk pressure (e), a group pressure term incorporating hydrogens into parent heavy atoms (f), and system volume (g). The harmonic restraint term used in the equilibration phase of the simulation is shown in (h), while various components of the system internal energy are illustrated in panels (i)→(l). Note that the more significant transitions in the dihedral energy term (denoted by ‘I’ and ‘II’ in panel l) correspond to conformational transitions evident in the structural parameters shown in the other supplementary figures (e.g., note the patterns of variability in the RMSD data shown in Figs. S3,4 and the striping pattern of matrix elements in S4).

Fig. S3: Stabilization and evolution of the κB DNA duplex structure over both short (ns) and long (μs) timescales. Successful equilibration and stabilization of the MD simulation over both short (top sub-panels) and long (bottom sub-panels) times was gauged by monitoring a variety of structural parameters over the course of the trajectory, including coordinate RMS deviations relative to (a) the energy-minimized starting structure that
served as input to the initial step of dynamics \((t_0)\), (b) the structure as it existed after 1 ns of equilibration \((t_{1000})\), (c) the average structure computed by calculating the mean coordinates of each atom in each frame of the production run, excluding the \(0\rightarrow 1\)-ns equilibration period \((i.e., \text{from} \: 1\rightarrow 1021 \: \text{ns}; \: \langle t_{1\rightarrow 1021} \rangle)\), (d) the \(\kappa B\) DNA element built into canonical B-form DNA structure, and (e) the \(\kappa B\) DNA element built into canonical A-form DNA. In each series of RMSD plots, values are shown for calculations taking into account various subsets of DNA atoms, as indicated. In addition to mass-weighting, the calculated “COM” RMSD values were computed using an additional multiplicative term linearly scaled against the distance of an atom to the duplex center-of-mass; therefore, the systematically lower values of this curve versus the non-COM curves show that the preponderance of structural variation occurs towards the termini of the double helix. Note that comparisons of RMSD traces for sliding averages computed over both narrow \((f)\) and broad \((g)\) time windows suggest the occurrence of some relatively long-lived states that more closely resemble A-form than B-form DNA (see the \(\approx\) 20 ns-wide peak regions in the lower panels of \(f\)).

**Fig. S4:** Conformational heterogeneity of the \(\kappa B\) DNA duplex across the ns \(\rightarrow\) \(\mu s\) timescales, as assessed by pairwise RMSD matrices. Similarities and differences in the structure of the \(\kappa B\) DNA were evaluated by calculating pairwise RMSD matrices over periods of \((a)\) 1-ns, \((b)\) 10-ns, \((c)\) 100-ns, and \((d)\) 1,020-ns. Due to the potentially vast size of the resultant datasets \((e.g., 10^{12}\) data points for a \(\mu s\) simulation sampled at ps time resolution), the larger matrices were sampled every 5-, 10-, or 50-ns, as indicated \((\delta t)\). Matrix elements are colored at linearly scaled values along a black \(\rightarrow\) red \(\rightarrow\) white gradient, as indicated in the color bars alongside each matrix, and dashed green lines in the lower-left corner of a given panel delineate the submatrix corresponding to the previous panel. Note that various aspects of the dynamics \((e.g.,\) harmonic positional restraints applied to DNA atoms over the first 400 ps of equilibration; Fig. S2h) are evident from the patterns of RMSD in \((a)\), and also that the large-scale conformational transitions which arise beyond 700 ns are revealed by the pattern of intensity of off-diagonal elements in \((d)\).

**Fig. S5:** Bundles of \(\kappa B\) DNA conformers and corresponding averaged structures over the course of one \(\mu s\). Individual snapshots along the entire trajectory were aligned to a common reference frame (selected based on criteria such as the RMSD results shown in Fig. S3), an overall average structure was then computed (excluding frames from the \(0\rightarrow 1\)-ns equilibration period), and then various average structures were derived by calculating mean coordinates for each DNA atom over particular slices of time, as indicated. For instance, an average was computed separately over the 1-ns equilibration period \((a)\), and all ten averages were computed over the \(10\times 100\)-ns bins ranging from the first snapshot of the production run \((t_{i,m})\) to the structure at the end of 1 \(\mu s\) of free dynamics \((t_{1,001-1,021})\). The final bundle group \((t_{1,000-1,021})\) corresponds to the DNA structure over the last 20 ns of the 1,020-ns production trajectory. DNA backbones within each bundle are drawn as thin lines, with colors scaled by progress along each period of time according to a red \((\text{start-of-bundle}) \rightarrow\) grey \(\rightarrow\) blue \((\text{end-of-bundle})\) gradient. Average structures over each time slice are illustrated as stick representations, with sugar rings colored red and blue for the two strands and hydrogen atoms omitted for the sake of clarity. The global helical axis (calculated using CURVES) is rendered as a green spline, and the bases of the \(\kappa B\) DNA element are drawn as CPK spheres (including hydrogens) scaled by atomic van der Waals radii. The two regions of greatest structural perturbation (indicated by arrows in panels \(i \rightarrow k\)) are also illustrated as CPK spheres: (i) the cytosine of the “barbed” \((G\text{-}C)\), terminus is colored lavender, (ii) the \((A\text{-}T)_{12}\) base pair which is the focal point of cross-strand intercalative stacking (XSIS) is colored yellow, and (iii) the neighboring \((A\text{-}T)_{13}\) thymine which is extruded from the helical stack in the base flipping transition is colored orange. Persistence of the bases in their novel/atypical positions in structures which were averaged over 100-ns periods of time is consistent with these being stable, long-lived conformational states rather than transient, metastable intermediates in the DNA dynamics.

**Fig. S6:** Evolution of \(\kappa B\) DNA helicoidal and stereochemical parameters over \(\mu s\) timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of \(\kappa B\) half sites. The multiple plots which comprise this figure illustrate the 3D structural evolution of the \(\kappa B\) DNA element over a greater than 1.0-\(\mu s\) time span, as quantified by the descriptors commonly used to describe the conformational properties of helical nucleic acids. In particular, the quantities plotted include the six rigid-body parameters (three translational and three rotational) which describe the geometry of individual base pairs with respect to a local (base pair-centered) reference frame: the translational parameters \((a)\) Shear \((S_x)\), Stretch \((S_y)\),...
and Stagger ($S_c$), as well as the angular values for the (b) Buckle ($\kappa$), Propeller twist ($\pi$), and Opening ($\sigma$). In addition, the six rigid-body parameters which define the position and orientation of consecutive base pairs relative to one another – i.e., the dinucleotide base pair step parameters – are provided as the (c) Shift ($D_x$), Slide ($D_y$), and Rise ($D_z$), as well as the angular parameters (d) Tilt ($\tau$), Roll ($\rho$), and Twist ($\omega$). Together with the helical parameters describing the position of a base pair with respect to the global helical reference frame (X-displacement, Inclination, etc.), these parameters provide a unique and complete description of the geometry of the double helix in terms of both local (dinucleotide step-based) and global (helical) reference frames. The evolution of a larger-scale helical parameter – the measured minor and major groove widths – is shown in (e). Mean ($\bar{\tau}$) and standard deviation ($\sigma$) values were computed over roughly the first half of the trajectory (from $50\rightarrow550$ ns, so as to exclude the XSIS and flipping portions), and are indicated in the legends for each panel. A schematic of the κB DNA element is provided alongside each of the panels $a \rightarrow e$, and A-T base pairs are colored light red so as to emphasize the A-rich nature of this specific κB DNA. The 9-bp κB recognition site is demarcated by grey lines, with the particular base pairs (numbered above) and base pair steps (numbered below and indicated by semicircular arcs) highlighted by a grey background and color-coded to match the data plots. For the sake of clarity, the data are plotted in scatter form (sampled every 5 ps), with locally-averaged values (using a 5-ns window) plotted as solid lines (except as noted in panels $f$ and $g$); values for ideal B-form DNA are indicated as thin grey lines for some of the parameters. Local minima/maxima within a 10-ns neighborhood are indicated as faint dashed lines for some data sets (e.g., $(A\rightarrow T)_{13}$ Shear in (a)). Finally, the geometric meaning of the local base pair and base pair step parameters are illustrated as thumbnail-sized schematics in the lower-left corner of each plot. As described in the text, the inferred coupling between cross-strand intercalative stacking (“XSIS”) and base pair flipping is reflected in the evolution of most local base pair and base pair step parameters, particularly in the 720→950 ns range. Analogous plots of base pair ($S_x$, $S_y$, $S_z$, $\kappa$, $\pi$, $\sigma$; panel $f$) and base pair step ($D_x$, $D_y$, $D_z$, $\tau$, $\rho$, $\omega$; panel $g$) parameters are provided for a region of the helix distal to the κB site (near $(T\rightarrow A)_5$; translational and angular parameters form the left and right halves of each sextuplet, respectively). Aside from occasional excursions (of up to $\approx50$-ns) toward $A$-form and intermediate $A/B$-like geometry, this region of the κB duplex exhibits predominantly ideal $B$-form-like behavior (values for canonical $A$- and $B$-form helices are shown as colored horizontal lines), thereby effectively providing an internal control on the μs-scale dynamics of the κB DNA element.

**Fig. S7:** Evolution of κB DNA local base pair geometry over both short (ns) and long (μs) timescales: Formation of “barbed” termini. The formation of a “barbed” minor groove-bound terminus can be described as the “peeling” away of a terminal nucleotide from its Watson-Crick base paired partner, followed by looping-back of the barbed base to form stable interactions (hydrogen-bonded and otherwise) with the parent strand on the floor of the proximal minor groove. Development of this novel conformation is distinct from the published base pair “fraying” events observed in MD simulations of nucleic acids and – because of its geometric properties – can be best quantified by local rigid-body base pair parameters which describe (i) the stretching of an ideal Watson-Crick base pair along the $y$-direction of the local base pair reference frame (the translational $Stretch$, $S_y$ parameter), and (ii) the rotational “opening” of one nucleotide of the base pair with respect to the other, in the plane of the base pair (the angular $Opening$, $\sigma$ parameter). The evolution of these parameters over both short ($S_y$) and long ($S_y$, $\sigma$) times is shown for the terminal $(G\rightarrow C)$, and $(A\rightarrow T)_{20}$ base pairs in panels (a) and (b), respectively. A schematic of the κB DNA element is provided alongside both sets of panels, and A-T base pairs are colored light red so as to emphasize the A-rich nature of this particular DNA. The 9-bp κB recognition site is demarcated by grey lines, with the particular base pairs for which data are plotted highlighted in yellow and color-coded to match the data plots. For the sake of clarity, the data are plotted in scatter form (sampled every 5 ps), with locally-averaged values (using a 5-ns window) plotted as solid lines; as in Fig. S6, faint dashed lines in the long-time sub-panels indicate local maxima/minima within a 10-ns neighborhood. The geometric meaning of the $Stretch$ and $Opening$ parameters are illustrated as thumbnail-sized schematics in the lower-left corner of each plot. Note that after an initial period of equilibration ($<1$-ns in upper-left panel of (a)), the 5' terminal $G\rightarrow C$ base pair evolves as a relatively stable structure for $\approx700$ ns, after which a systematic and high-amplitude deviation in both the $S_y$ and $\sigma$ parameters marks the onset of the barbed structure. This new structure persists for several hundreds of ns, and is apparently capable of re-forming after a roughly 20-ns excursion into a non-barbed state ($\approx890$ ns). Consistent with previously observed dynamical behavior of G-C versus A-T termini, the $(G\rightarrow C)_1$ pair exhibits only small-scale transient “fraying” events over the first 700 ns of the trajectory (a), whereas the 3’
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terminal (A-T)$_{20}$ pair displays much more stochastic behavior characterized by higher frequency and amplitude fluctuations of both the Stretch and Opening parameters (b).

**Fig. S8. DNA backbone dynamics at the junction of κB half sites: Equilibrium sampling of canonical $A$-, $B$-, and $TA$-like helical morphologies.** Values of the phosphate-based $Z_P/Z_P(h)$ metric are shown for base pair steps 13 (a) and 14 (b), which overlap the XSIS/flipping locus between κB half sites. As in Fig. 8, data values over the full $\mu$s are plotted as temporally-colored points. The maximal extent of sampling is illustrated by the convex hull (orange line), and regions characteristic of $A$, $B$, and $TA$-like DNA (arrows in a) are demarcated by dashed cyan lines.
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Supp. Fig. S1: Overview of the κB DNA simulation system: Sequence, structure, and hydrodynamic properties.
Molecular dynamics of κB DNA

Supp. Fig. S1: Overview of the κB DNA simulation system: Sequence, structure, and hydrodynamic properties.
(continued)

d) minor groove-bound “barbed” terminus (Fig. 8)
cross-strand intercalative stacking of nucleotides (Fig. 5)
base-flipping of Thy2,8 (Fig. 7)
degenerate rotational modes of cylindrically-symmetric duplex

e) Rotational relaxation properties of the κB DNA element
Supp. Fig. S2: Thermodynamic equilibration and stabilization of the κB DNA system.

(a) Total Energy of System
(b) Potential Energy of System
(c) Kinetic Energy of System
(d) Temperature of System
(e) Pressure of System
(f) Group Pressure of System
Supp. Fig. S2: Thermodynamic equilibration and stabilization of the κB DNA system. (continued)
Supp. Fig. S3: Stabilization and evolution of the κB DNA duplex structure over both short (ns; top) and long (μs; bottom) timescales.

a) Short-time RMSDs referred to initial structure ($t_0$)

Long-time RMSDs referred to initial structure ($t_0$)
Supp. Fig. S3: Stabilization and evolution of the κB DNA duplex structure over both short (ns; top) and long (μs; bottom) timescales. (continued)

b) Short-time RMSDs referred to equilibrated structure ($t_{1000}$)

Long-time RMSDs referred to equilibrated structure ($t_{1000}$)
Supp. Fig. S3: Stabilization and evolution of the κB DNA duplex structure over both short (ns; top) and long (μs; bottom) timescales. (continued)

c) Short-time RMSDs referred to average structure ($\langle t_{1-1021ns} \rangle$)
Supp. Fig. S3: Stabilization and evolution of the κB DNA duplex structure over both short (ns; top) and long (μs; bottom) timescales.

(continued)

d) Short-time RMSDs referred to canonical B-form DNA

![Short-time RMSDs](image)

Long-time RMSDs referred to canonical B-form DNA

![Long-time RMSDs](image)
Supp. Fig. S3: Stabilization and evolution of the κB DNA duplex structure over both short (ns; top) and long (μs; bottom) timescales.

(continued)

e) Short-time RMSDs referred to canonical A-form DNA

Long-time RMSDs referred to canonical A-form DNA
Supp. Fig. S3: Stabilization and evolution of the κB DNA duplex structure over both short (ns; top) and long (μs; bottom) timescales.

(continued)

**f)** Comparison of long-time RMSDs, **narrow time windows**

![Graph showing comparison of long-time RMSDs, narrow time windows](image-url)
Supp. Fig. S3: Stabilization and evolution of the κB DNA duplex structure over both short (ns; top) and long (μs; bottom) timescales. (continued)

\[ g) \] Comparison of long-time RMSDs, **broad time windows**

![Graph showing comparison of long-time RMSDs](image)
Supp. Fig. S4: Conformational heterogeneity of the κB DNA duplex across the ns → μs timescales, as assessed by pairwise RMSD matrices.

a) 1.0 ns ($t_0 \rightarrow t_{1-ns}$; $\delta t = 1$ ps)

b) 10.0 ns ($t_0 \rightarrow t_{10-ns}$; $\delta t = 5$ ps)
Supp. Fig. S4: Conformational heterogeneity of the κB DNA duplex across the ns → μs timescales, as assessed by pairwise RMSD matrices.
(continued)

\( c) \ 100.0 \text{ ns } (t_0 \rightarrow t_{100\text{-ns}}; \delta t = 10\text{ps}) \)

\( d) \ 1,020.0 \text{ ns } (t_1 \rightarrow t_{1021\text{-ns}}; \delta t = 50\text{ps}) \)
Supp. Fig. S5: Bundles of κB DNA conformers and corresponding averaged structures over the course of 1 µs.

a) $t_0 \rightarrow t_{1\text{-ns}}$

b) $t_1 \rightarrow t_{101\text{-ns}}$

c) $t_{101} \rightarrow t_{201\text{-ns}}$

d) $t_{201} \rightarrow t_{301\text{-ns}}$

e) $t_{301} \rightarrow t_{401\text{-ns}}$

f) $t_{401} \rightarrow t_{501\text{-ns}}$

g) $t_{501} \rightarrow t_{601\text{-ns}}$

h) $t_{601} \rightarrow t_{701\text{-ns}}$
Supp. Fig. S5: Bundles of κB DNA conformers and corresponding averaged structures over the course of 1 μs.

(continued)

i) $t_{701} \rightarrow t_{801}$-ns  

j) $t_{801} \rightarrow t_{901}$-ns  

k) $t_{901} \rightarrow t_{1,001}$-ns  

l) $t_{1,001} \rightarrow t_{1,020}$-ns
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites.

a) $5'GGGGTTTAAAGAAAAATTCC'3'$

Long-time evolution of $\text{BPs}_{10\rightarrow 15}$ local base-pair parameter: Shear

Long-time evolution of $\text{BPs}_{10\rightarrow 15}$ local base-pair parameter: Stretch
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites. (cont’d)

a) 5′ G G G T T T A A A A G A A A T T C C C A A A T T T C T T T A A G G T C T 3′

Long-time evolution of BPs: local base-pair parameter: Stagger

b) Long-time evolution of BPs: local base-pair parameter: Buckle

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Supp. Fig. S6 (cont’d)
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites. (cont’d)

Long-time evolution of $\text{BPs}_{10\rightarrow 15}$ local base-pair parameter: **Propeller twist**

Long-time evolution of $\text{BPs}_{10\rightarrow 15}$ local base-pair parameter: **Opening**
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites. (cont’d)

C) $5^\prime$GGGTTTAAA$G$ A A A T T$G$CCCAAATTT$C$ T T T A A$C$ GGT CT$C$

Long-time evolution of Steps $10 \rightarrow 14$ local base pair step parameter: **Shift**

Long-time evolution of Steps $10 \rightarrow 14$ local base pair step parameter: **Slide**
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: *Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites.* (cont’d)

c) 5' GGGTTTAAA 3'
    CCAAAATTTCCTTAAGGCTCT

Long-time evolution of Steps 10–14 local base pair step parameter: Rise

![Graph showing rise over time for different sequences](image)

Long-time evolution of Steps 10–14 local base pair step parameter: Tilt

![Graph showing tilt over time for different sequences](image)
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites. (cont’d)

d) 5' G G G T T T A A A G A A A T T C C A G A 3'
C C C A A A T T T C T T T A A G G T C T

Long-time evolution of Steps 10→14 local base pair step parameter: Roll

Long-time evolution of Steps 10→14 local base pair step parameter: Twist
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites. (cont’d)

e)  

Long-time evolution of Steps 10→14 local base pair steps: **Minor groove**

![Minor groove plot](image)

Long-time evolution of Steps 10→14 local base pair steps: **Major groove**

![Major groove plot](image)
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites. (cont’d)

f) Long-time evolution of local base-pair parameter: Shear

Long-time evolution of local base-pair parameter: Buckle

Long-time evolution of local base-pair parameter: Stretch

Long-time evolution of local base-pair parameter: Propeller twist

Long-time evolution of local base-pair parameter: Stagger

Long-time evolution of local base-pair parameter: Opening
Supp. Fig. S6: Evolution of κB DNA helicoidal and stereochemical parameters over μs timescales: Coupling between cross-strand intercalative stacking of nucleotides and spontaneous base flipping at the junction of κB half sites. (cont’d)

Long-time evolution of local base pair step parameter: **Shift**

Long-time evolution of local base pair step parameter: **Tilt**

Long-time evolution of local base pair step parameter: **Slide**

Long-time evolution of local base pair step parameter: **Roll**

Long-time evolution of local base pair step parameter: **Rise**

Long-time evolution of local base pair step parameter: **Twist**
Supp. Fig. S7: Evolution of κB DNA local base pair geometry over both short (ns) and long (μs) timescales: Formation of “barbed” termini.

a) 5' C G G T T T A A A G A A A T T C C ' C A G A 3'
   C C A A A T T T T T T A A G G T C T

Short-time evolution of $bp_1$ (G-C) local parameter: Stretch

Long-time evolution of $bp_1$ (G-C) local parameter: Opening

Long-time evolution of $bp_1$ (G-C) local parameter: Stretch

transient fraying events
Supp. Fig. S7: Evolution of ςB DNA local base pair geometry over both short (ns) and long (μs) timescales: Formation of “barbed” termini. (cont’d)

b) $S^G G G T T T A A A G A A A T T C C A G A C C C A A A T T T C T T T A A G G T C T^3'$

Short-time evolution of $b_{20}$ (A-T) local parameter: Stretch

Long-time evolution of $b_{20}$ (A-T) local parameter: Opening

Long-time evolution of $b_{20}$ (A-T) local parameter: Stretch
Supp. Fig. S8. DNA backbone dynamics at the junction of κB half sites: Equilibrium sampling of canonical $A\sim$, $B\sim$, and $TA\sim$-like helical morphologies.

a) Step 13, AT/AT

b) Step 14, TT/AA
Supplementary Animations

Video animations illustrating interesting conformational transitions over the course of the μs-long trajectory are available at the following URLs (animations utilize the same coloring conventions and graphical representation schemes as in the main Figures and other Supporting Information):

a) http://mccammon.ucsd.edu/~cmura/DNA/XStrandStacking.mpg – A video illustration of the cross-strand intercalative stacking (“XSIS”) that first appears in the ≈ 0.8 μs region of the trajectory (Figs. 3–6, S5, S6). This transition is characterized by severe disruption of the (A·T)$_{12}$ Watson-Crick base pair, such that the constituent bases (Ade$_{1,12}$ and Thy$_{2,9}$) shear apart and assume a coaxial rather than coplanar arrangement – i.e., they become stacked upon one another, with Ade$_{1,12}$ staggered towards the 5’ direction and the cross-strand partner Thy$_{2,9}$ translated towards the 3’ direction (with respect to the 5’→3’ path of the parent strand along the global helical axis). This large-scale structural transition is closely coupled to the base flipping of thymine at the adjacent (A·T)$_{13}$ base pair.

b) http://mccammon.ucsd.edu/~cmura/DNA/BaseFlipping.mpg – This animation visualizes the spontaneous Thy$_{2,8}$ base flipping event that arises near the end of the μs-long trajectory (Figs. 3, 4, 7, S5, S6). Occurring well beyond 0.9 μs and immediately adjacent to the sheared/XSIS (A·T)$_{12}$ base pair, the thymine base that is the focal point of this transition can be seen to swivel entirely out of the double helical stack, thereby eliminating the (A·T)$_{13}$ base pair and forming an abasic (apyrimidinic) lesion. Notably, the extruded Thy$_{2,8}$ (together with Ade$_{1,13}$) forms the junction between the AGAA and TTCC κB half sites which together comprise the nonameric $^9$AGAAN$^9$TTCC$^3$ DNA recognition element for (c-Rel)$_2$ and related members of this subfamily of NF-κB transcription factors.

c) http://mccammon.ucsd.edu/~cmura/DNA/BarbedTerminus.mpg – This illustration shows the formation of a “barbed” structure at the 5’ terminus of the GGGT··· sequence (sense) strand (Figs. 4, 8, S7). This peculiar structure is characterized by a “peeling”-away of the cytosine base from the terminal (G·C)$_1$ guanine, followed by stable positioning of the Cyt pyrimidyl moiety into the proximal minor groove of its parent strand (antisense strand in Fig. S1c). This barbed terminus (i) is distinct from the documented base pair “fraying” events observed in ns-scale MD simulations of helical nucleic acids, (ii) recurs at long times (>0.7 μs) in the trajectory, and (iii) stably persists for lengths of time on the order of 100 ns (as seen in Figs. 8, S7, and as evidenced by persistence of the barbed conformation in structures averaged over 100 ns-wide windows; Fig. 4c-f).

d) http://mccammon.ucsd.edu/~cmura/DNA/Overview.mpg – Providing an overview of the entire trajectory, this animation depicts the dynamics of the κB DNA from two views (transverse and axial) over the course of 1,021 ns. The animation is rather lengthy (3.5 min); viewers are encouraged to advance to at least the half-way point (~ 0.5 μs), as that is nearer to the onset of the interesting barbing, XSIS, and base flipping transitions.