

Influence of an intrinsically disordered region on protein domains revealed by NMR-based electrostatic potential measurements

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Supporting Information Placeholder

ABSTRACT: Many human proteins possess intrinsically disordered regions containing consecutive aspartate or glutamate residues ('D/E repeats'). Approximately a half of them are DNA/RNA-binding proteins. In this study, using nuclear magnetic resonance (NMR) spectroscopy, we investigated the electrostatic properties of D/E repeats and their influence on folded domains within the same protein. Local electrostatic potentials were directly measured for the HMGB1 protein, its isolated D/E repeats, and the DNA-binding domains by NMR. The data provide quantitative information about the electrostatic interactions between distinct segments of HMGB1. Due to the interactions between the D/E repeats and the DNA-binding domains, local electrostatic potentials of the DNA-binding domains within the full-length HMGB1 protein were largely negative despite the presence of many positively charged residues. Our NMR data on counterions and electrostatic potentials show that the D/E repeats and DNA have similar electrostatic properties and compete for the DNA-binding domains. The competition promotes dissociation of the protein-DNA complex and influences the molecular behavior of the HMGB1 protein. These effects may be general among the DNA/RNA-binding proteins with D/E repeats.

Hundreds of human proteins have low-complexity sequences of consecutive aspartate (D) or glutamate (E) residues, which are referred to as D/E repeats (examples shown in Figure 1A).^{1,2} Approximately 50% of proteins containing D/E repeats are DNA/RNA-binding proteins. For some of them, the D/E repeats interact with the DNA-binding domains within the same polypeptide chain and diminish the association with DNA, causing autoinhibition.³⁻⁸ Other D/E repeats can exhibit chaperone-like activity.^{9,10} Currently, however, very little is known about the physicochemical properties of D/E repeats. In this paper, using NMR spectroscopy, we demonstrate that D/E repeats have electrostatic properties similar to those of DNA and electrostatically influence the

DNA-binding domains via intramolecular interactions, competing with DNA.

We first investigated the electrostatic properties of the 31-residue peptide containing D/E repeats of 30 residues that correspond to the C-terminal tail (residues 186-215) of the human HMGB1 protein. We referred to this peptide as DERT30. Figure 1B shows ¹H-¹⁵N heteronuclear HSQC¹¹ and direct ¹³C-detected CON¹² spectra recorded for ¹³C/¹⁵N-labeled DERT30. Despite the low complexity of its sequence, many cross peaks in these spectra were well isolated owing to sharp ¹⁵N and ¹³C=O resonances of the IDR. Through direct ¹³C-detected¹³ and ¹H-detected¹⁴ NMR experiments, we assigned each isolated signal from the D/E repeats (see Figures S1-S2 and Materials and Methods described in the Supporting Information [SI]). Negative heteronuclear [¹H-]¹⁵N NOE values (Figure 1C) and small ¹⁵N chemical shift anisotropy (CSA) / ¹⁵N-¹H dipole-dipole (DD) cross-correlation rates (Figure S3) for backbone NH groups¹⁵ clearly indicate that D/E repeats are structurally disordered.

Using NMR data of solvent paramagnetic relaxation enhancement (PRE) arising from aminomethyl-PROXYL (charge, +1e) and carbamoyl-PROXYL (neutral) as paramagnetic cosolutes, we measured effective near-surface electrostatic potentials ϕ_{ENS} around DERT30. Since our group developed the NMR method to measure ϕ_{ENS} potentials in 2021,¹⁶ we and others have applied it to various biomolecules, as recently reviewed.¹⁷ In the current study, we measured the solvent PRE Γ_2 rates on backbone ¹H _{α} nuclei (Figure S4) to determine ϕ_{ENS} potentials. We analyzed data for ¹H _{α} nuclei instead of ¹H_N nuclei because backbone amide groups of a disordered polypeptide at neutral pH typically undergo rapid hydrogen exchange,¹⁸ which may adversely impact solvent PRE rates (Γ_2). Figure 1D shows the effective near-surface electrostatic potentials ϕ_{ENS} determined from the solvent PRE data. Interestingly, the overall magnitude of the negative potentials for the D/E repeat was even larger than that of potentials previously measured for the 15-bp DNA¹⁹ under the same conditions.

protein and the $\Delta 30$ variant lacking the D/E repeats. Most residues of the DNA-binding domains exhibited positive potentials in the $\Delta 30$ variant. By contrast, the presence of the D/E repeats in the full-length protein caused negative ϕ_{ENS} potentials for many residues. These results illuminate the strong electrostatic influence of the D/E repeats on the DNA-binding domains of HMGB1.

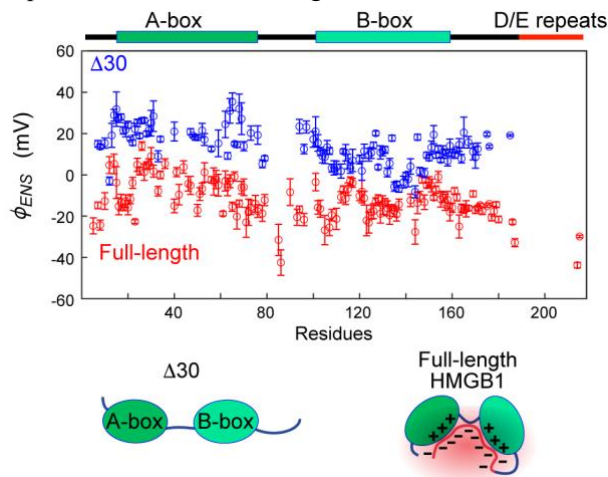


Figure 3. Effective near-surface potentials ϕ_{ENS} measured for the full-length HMGB1 protein (red) and the $\Delta 30$ variant lacking the D/E repeats (blue). Solvent PRE data used to determine the ϕ_{ENS} potentials are shown in Figure S7.

Through NMR experiments, we examined competition between D/E repeats and DNA for the DNA-binding domains. We recorded ^1H - ^{15}N HSQC spectrum for the complex of ^{15}N -labeled DERT30 and the unlabeled $\Delta 30$ variant of HMGB1. Due to the interaction with the DNA-binding domains, the spectrum recorded for the ^{15}N -labeled DERT30 was significantly different from the spectrum for the free state, but the signal dispersion remained poor (Figure 4A left). This suggests that the D/E repeats interacting with the $\Delta 30$ variant remain as a random coil, which is also supported by $^{13}\text{C}_\alpha$ chemical shifts (Figure S8). To examine competition between D/E repeats and DNA, we used a four-way junction DNA (32 base pairs) named J1,²⁶ which exhibits high affinities for the full-length HMGB1^{8,27} and the $\Delta 30$ variant (the binding affinity data are shown in Figure S9). When unlabeled J1 was added to the solution of the complex of ^{15}N DERT30 and the unlabeled $\Delta 30$ variant, the signals from ^{15}N -labeled DERT30 moved toward the chemical shifts for the free state (Figure 4A middle). These NMR data indicate that DNA and the D/E repeats compete for the DNA-binding domains of HMGB1.

The molecular behavior of HMGB1 is significantly influenced by the competition between the D/E repeats and DNA. As shown in Figure 4B, the D/E repeats cause 5-20-fold faster dissociation of HMGB1 from DNA, clearly interfering with the binding of the structured domains with DNA. The $\Delta 30$ variant lacking the D/E repeats exhibited a linear increase of the apparent rate

constant for dissociation (k_{off}^{app}) as a function of the unlabeled DNA concentration. On the contrary, the full-length HMGB1 protein exhibited a decrease in k_{off}^{app} . Both behaviors can be explained using the kinetic models described in SI Section 3 (see also Figure S12). The linear increase observed for $\Delta 30$ is indicative of the so-called intersegment transfer, whereby a protein transfers from one DNA to another through a transient intermediate bridging two DNA molecules.²⁸⁻³⁰ The bridging intermediate can be formed when one of the two DNA-binding domains is dissociated from DNA and captures another DNA molecule. By contrast, the full-length protein did not show any sign of intersegment transfer. As schematically drawn in Figure 4B, the D/E repeats can engage the detached domain (A-box or B-box), thus precluding the formation of the bridging intermediate, and facilitate macroscopic dissociation of the protein from DNA.

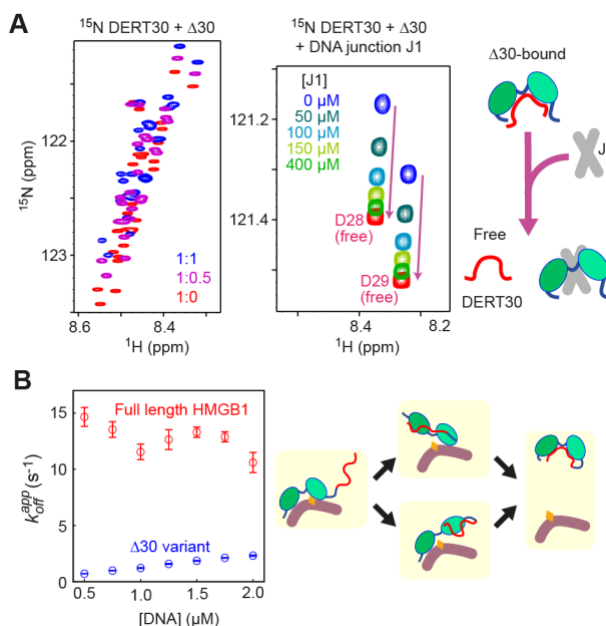


Figure 4. D/E repeats compete with DNA for the DNA-binding domains of HMGB1. (A) Change of ^1H - ^{15}N NMR spectra recorded for ^{15}N -labeled DERT30. The left panel shows overlaid spectra recorded for $\Delta 30$ -bound (blue) and free (red) states of ^{15}N DERT30 (0.2 mM). When the four-way junction DNA J1 (32 base pairs) was added to the solution of the DERT30- $\Delta 30$ complex, the NMR signals from DERT30 were found to shift toward those from the free state (right panel). Simulations of this effect using the Bloch-McConnell equation are shown in Figure S10. (B) Apparent rate constant for dissociation (k_{off}^{app}) of the full-length HMGB1 or its $\Delta 30$ variant protein from cisplatin-modified DNA. They were measured through stopped-flow fluorescence experiments in which the fluorescence anisotropy was monitored immediately after a solution of the complex (10 nM) of the protein and fluorescence-labeled DNA (20 base pairs) containing a site-specific modification by cisplatin was mixed with a solution of the same DNA with no fluorescence label (0.5-2.0 μM) (Figure S11). The theoretical relationship between the apparent and intrinsic

dissociation rate constants are described in the SI (see also Figure S12).

Overall, our data suggest that despite the completely different chemical structures, D/E repeats can serve as DNA mimetic in terms of electrostatics and strongly influence the molecular properties of DNA-binding proteins. Our previous study showed that the intramolecular interactions between D/E repeats and the DNA-binding domains let the protein molecules avoid the distraction of non-target DNA and thereby accelerate the target search process.³¹ Because D/E repeats are conformationally more flexible than double-stranded DNA, electrostatic repulsive forces from DNA may induce conformational transitions of the proteins from an autoinhibited state to an uninhibited state, allowing the proteins to bind to DNA.³¹ Through a reverse process, D/E repeats can also promote dissociation of the protein from DNA (Figure 4B). In this manner, D/E repeats can mobilize the protein bound to DNA and facilitate translocation from one site to another.

In conclusion, our current study shows that D/E repeats have DNA-like electrostatic properties and act as DNA mimicry that affects the molecular properties of a DNA-binding protein. The direct measurement of electrostatic potentials by NMR reveals the electrostatic influence of the D/E repeats on the DNA-binding domains. The D/E repeats compete with DNA for the DNA-binding domains and facilitate dissociation of the protein from DNA. A similar mechanism might also work for RNA-binding proteins containing D/E repeats. It is likely that the approach using NMR-based measurement of electrostatic potentials will be useful for characterization of many other proteins containing intrinsically disordered regions (IDRs) and provide quantitative information about electrostatic interactions between IDRs and folded domains.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website.

Materials and Methods; Figures S1-S12; and Table S1 (PDF).

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REFERENCES

- (1) Bigman, L. S.; Iwahara, J.; Levy, Y. Negatively Charged Disordered Regions are Prevalent and Functionally Important Across Proteomes. *J Mol Biol* **2022**, *434*, 167660.
- (2) Shukla, S.; Lazarchuk, P.; Pavlova, M. N.; Sidorova, J. M. Genome-wide survey of D/E repeats in human proteins uncovers their instability and aids in identifying their role in the chromatin regulator ATAD2. *iScience* **2022**, *25*, 105464.
- (3) Stott, K.; Watson, M.; Howe, F. S.; Grossmann, J. G.; Thomas, J. O. Tail-mediated collapse of HMGB1 is dynamic and occurs via differential binding of the acidic tail to the A and B domains. *J Mol Biol* **2010**, *403*, 706.
- (4) Watson, M.; Stott, K.; Thomas, J. O. Mapping intramolecular interactions between domains in HMGB1 using a tail-truncation approach. *J Mol Biol* **2007**, *374*, 1286.
- (5) Wiebe, M. S.; Nowling, T. K.; Rizzino, A. Identification of novel domains within Sox-2 and Sox-11 involved in autoinhibition of DNA binding and partnership specificity. *J Biol Chem* **2003**, *278*, 17901.
- (6) Wang, X.; Greenblatt, H. M.; Bigman, L. S.; Yu, B.; Pletka, C. C.; Levy, Y.; Iwahara, J. Dynamic Autoinhibition of the HMGB1 Protein via Electrostatic Fuzzy Interactions of Intrinsically Disordered Regions. *J Mol Biol* **2021**, *433*, 167122.
- (7) Ueshima, S.; Nagata, K.; Okuwaki, M. Internal Associations of the Acidic Region of Upstream Binding Factor Control Its Nucleolar Localization. *Mol Cell Biol* **2017**, *37*, e00218.
- (8) Wang, X.; Mayorga-Flores, M.; Bien, K. G.; Bailey, A. O.; Iwahara, J. DNA-mediated proteolysis by neutrophil elastase enhances binding activities of the HMGB1 protein. *J Biol Chem* **2022**, *298*, 102577.
- (9) Huang, L.; Agrawal, T.; Zhu, G.; Yu, S.; Tao, L.; Lin, J.; Marmorstein, R.; Shorter, J.; Yang, X. DAXX represents a new type of protein-folding enabler. *Nature* **2021**, *597*, 132.
- (10) Sitron, C. S.; Hartl, F. U. A new way of D/Ealing with protein misfolding. *Mol Cell* **2021**, *81*, 4114.
- (11) Yuwen, T.; Skrynnikov, N. R. CP-HISQC: a better version of HSQC experiment for intrinsically disordered proteins under physiological conditions. *J Biomol NMR* **2014**, *58*, 175.
- (12) Gil, S.; Hošek, T.; Solyom, Z.; Kümmerle, R.; Brutscher, B.; Pierattelli, R.; Felli, I. C. NMR Spectroscopic Studies of Intrinsically Disordered Proteins at Near-Physiological Conditions. *Angew Chem Int Ed* **2013**, *52*, 11808.
- (13) Felli, I. C.; Pierattelli, R. ¹³C Direct Detected NMR for Challenging Systems. *Chem Rev* **2022**, *122*, 9468.
- (14) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G., III; Rance, M.; Skelton, N. J. *Protein NMR Spectroscopy: Principles and Practice*; 2 ed.; Elsevier Academic Press: Burlington, 2007.
- (15) Palmer, A. G., 3rd NMR probes of molecular dynamics: overview and comparison with other techniques. *Annu Rev Biophys Biomol Struct* **2001**, *30*, 129.
- (16) Yu, B.; Pletka, C. C.; Pettitt, B. M.; Iwahara, J. De novo determination of near-surface electrostatic potentials by NMR. *Proc Natl Acad Sci U S A* **2021**, *118*, e2104020118.
- (17) Iwahara, J.; Pettitt, B. M.; Yu, B. Direct measurements of biomolecular electrostatics through experiments. *Curr Opin Struct Biol* **2023**, *82*, 102680.
- (18) Dyson, H. J.; Wright, P. E. NMR illuminates intrinsic disorder. *Curr Opin Struct Biol* **2021**, *70*, 44.
- (19) Yu, B.; Wang, X.; Iwahara, J. Measuring Local Electrostatic Potentials Around Nucleic Acids by Paramagnetic NMR Spectroscopy. *J Phys Chem Lett* **2022**, *13*, 10025.
- (20) Grzesiek, S.; Bax, A. The importance of not saturating H₂O in protein NMR. Application to sensitivity enhancement and NOE measurements. *J Am Chem Soc* **1993**, *115*, 12593.
- (21) Yu, B.; Bien, K. G.; Pletka, C. C.; Iwahara, J. Dynamics of Cations around DNA and Protein as Revealed by ²³Na Diffusion NMR Spectroscopy. *Anal Chem* **2022**, *94*, 2444.

- (22) Jurrus, E.; Engel, D.; Star, K.; Monson, K.; Brandi, J.; Felberg, L. E.; Brookes, D. H.; Wilson, L.; Chen, J.; Liles, K. *et al.* Improvements to the APBS biomolecular solvation software suite. *Protein Sci* **2018**, *27*, 112.
- (23) Fogolari, F.; Brigo, A.; Molinari, H. The Poisson-Boltzmann equation for biomolecular electrostatics: a tool for structural biology. *J Mol Recognit* **2002**, *15*, 377.
- (24) Grochowski, P.; Trylska, J. Continuum molecular electrostatics, salt effects, and counterion binding—A review of the Poisson–Boltzmann theory and its modifications. *Biopolymers* **2008**, *89*, 93.
- (25) Netz, R. R.; Orland, H. Beyond Poisson-Boltzmann: Fluctuation effects and correlation functions. *Eur Phys J E* **2000**, *1*, 203.
- (26) Kallenbach, N. R.; Ma, R.-I.; Seeman, N. C. An immobile nucleic acid junction constructed from oligonucleotides. *Nature* **1983**, *305*, 829.
- (27) Troisi, M.; Klein, M.; Smith, A. C.; Moorhead, G.; Kebede, Y.; Huang, R.; Parker, E.; Herrada, H.; Wade, E.; Smith, S. *et al.*

Conformation and protein interactions of intramolecular DNA and phosphorothioate four-way junctions. *Exp Biol Med (Maywood)* **2021**, *246*, 707.

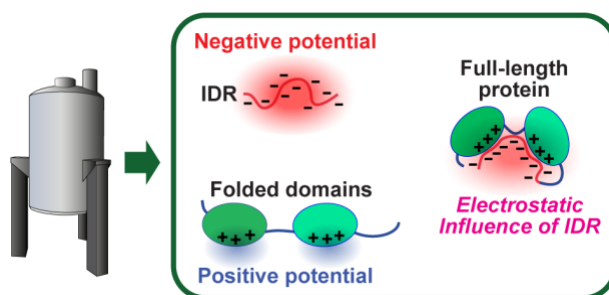
(28) Itoh, Y.; Murata, A.; Takahashi, S.; Kamagata, K. Intrinsically disordered domain of tumor suppressor p53 facilitates target search by ultrafast transfer between different DNA strands. *Nucleic Acids Res* **2018**, *46*, 7261.

(29) Esadze, A.; Iwahara, J. Stopped-flow fluorescence kinetic study of protein sliding and intersegment transfer in the target DNA search process. *J Mol Biol* **2014**, *426*, 230.

(30) Doucleff, M.; Clore, G. M. Global jumping and domain-specific intersegment transfer between DNA cognate sites of the multidomain transcription factor Oct-1. *Proc Natl Acad Sci U S A* **2008**, *105*, 13871.

(31) Wang, X.; Bigman, L. S.; Greenblatt, H. M.; Yu, B.; Levy, Y.; Iwahara, J. Negatively charged, intrinsically disordered regions can accelerate target search by DNA-binding proteins. *Nucleic Acids Res* **2023**, *51*, 4701.

TOC graphic



SUPPORTING INFORMATION

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1. Materials and Methods

Preparation of DERT30

A pET50b-derivative plasmid containing a codon-optimized synthetic gene of the His₆-tagged SUMO-DERT30 fusion protein at NdeI/PacI sites was purchased from GenScript, Inc. The plasmid was used for transformation of the *Escherichia coli* strain BL21 (DE3). A transformed clone was cultured at 37°C in a total of 2-L media containing 30 µg/ml kanamycin (as the selection marker), 1 g/L ¹⁵NH₄Cl (as the sole nitrogen source) and 2 g/L ¹³C-glucose (as the sole carbon source). When the optical density at 600 nm reached 0.8, expression of the His₆-tagged SUMO-DERT30 fusion protein was induced by adding 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Immediately after the addition of IPTG, the culture temperature was changed to 18°C and the culture was continued at this temperature for 16 hours. The cells were harvested through a centrifugation at 4,000 × g and suspended in a ~80 ml buffer of 20 mM Tris•HCl, 1 mM EDTA, 500 mM NaCl, 2 mM dithiothreitol (DTT) and 5% Glycerol at pH 7.5. The buffer also contained a Roche Complete protease inhibitors cocktail (2 tablets per 100 ml). The suspended cells were stored at -70°C until use. For isolation of the His₆-tagged SUMO-DERT30 fusion protein, the cells were defrosted and broken by sonication. After centrifugation of the lysate at 30,000 × g for 20 minutes at 4°C, the supernatant was loaded onto a 20-ml His Prep FF column equilibrated with a buffer of 50 mM Tris•HCl, 500 mM NaCl, 5 mM imidazole and 5% Glycerol at pH7.5. The column was washed with 50 ml of a buffer of Tris•HCl, and then, the His₆-tagged SUMO-DERT30 fusion protein was eluted by a gradient of 5-400 mM imidazole in a buffer of Tris•HCl. The isolated fusion protein was cleaved by the His₆-tagged Ulp1c SUMO protease. To remove the protease and the resultant His₆-SUMO tag, the reaction mixture was passed through a 20-ml His Prep FF column. The flow-through was collected and loaded onto a Resource-Q column equilibrated with a buffer of 50 mM Tris•HCl and 1 mM EDTA at pH7.5. DERT30 was eluted through a gradient of 0-1500 mM NaCl in the same buffer. The peptide was quantified using UV absorbance at 280 nm along with the extinction coefficient of 1,490 M⁻¹ cm⁻¹, which was predicted by the ExPasy ProtParam server (<https://web.expasy.org/cgi-bin/protparam>).

Preparation of HMGB1 and its Δ30 variant

¹⁵N-labeled HMGB1 and its Δ30 variant were expressed in *Escherichia coli* strain BL21 (DE3) and purified using ion-exchange and size-exclusion columns as previously described.¹ The purified

proteins were lyophilized and kept at -20°C until use. The HMGB1 and its $\Delta 30$ variant proteins were quantified using UV absorbance at 280 nm along with an extinction coefficient of 21,555 M⁻¹ cm⁻¹, which was predicted by the ExPasy ProtParam server.

Preparation of DNA

All DNA strands used in the current study were chemically synthesized and were purchased from Integrated DNA Technologies, Inc. The four-way junction DNA J1 was composed of four oligonucleotides designed by Kallenbach et al.²: J1A, CGCAATCCTGAGCACG; J1B, CGTGCTCACCGAATGC; J1C, GCATTCTGGACTATGGC; and J1D, GCCATAGTGGATTGCG. The FAM moiety was attached to the 5'-terminus of the J1A strand during DNA synthesis. Each strand was purified using Mono-Q anion exchange chromatography. After annealing of an equimolar mixture of the four strands, the four-way junction J1 was further purified by Mono-Q anion exchange chromatography. The 20-bp DNA with cisplatin modification was prepared as previously described.¹ Briefly, a 20-mer DNA strand containing single GpG site (CTCTGGACCTTCCTTTCTTC; denoted GG20) was platinated by addition of activated cisplatin solution. The reaction product was purified by Resource-Q anion exchange chromatography. After annealing of complimentary strands, the duplex was further purified by Resource-Q column. For fluorescence studies, FAM was attached to the 5'-terminus of GG20.

¹H/¹³C/¹⁵N NMR resonance assignment

The ¹H, ¹³C, ¹⁵N resonances of the HMGB1 and $\Delta 30$ variant proteins under the current experimental conditions were previously assigned.³ The ¹H, ¹³C, and ¹⁵N resonances of DERT30 were assigned using a 520 μ l solution of 0.3 mM ¹³C/¹⁵N-labeled DERT30 in a buffer of 100 mM NaCl, 10 mM DMSO, 20 mM Tris•acetate, and 5% D₂O at pH 7.5. An Amicon Ultra-4 device (Millipore) with a molecular weight cut-off at 3 kDa was used for concentrating and buffer equilibration. Although a slight leak of DERT30 was found due to its molecular weight (3.9 kDa) close to the cut-off, the vast majority remained in the upper compartment of the centrifugal filter. For the backbone ¹H/¹³C/¹⁵N resonance assignment, ¹H-¹⁵N HISQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, HN(COCA)HA, HN(COCA)CB, CON, CO(CA)N, and CA(CO)N spectra were recorded.⁴ In addition to a standard CON spectrum, Asp(D)/Asn(N)-selective and Glu(E)/Gln(Q)-selective CON spectra⁵ were also recorded to confirm amino-acid types.⁶ For the direct ¹³C-detected spectra, the virtual ¹³C-¹³C decoupling⁶ was used, and the obtained data were processed with the 'splitcomb' command of Bruker TopSpin software. Because the ¹⁵N chemical shift distribution of the D/E repeats is very narrow, a sufficiently high resolution in the ¹⁵N dimensions was achieved only through a non-constant-time type ¹⁵N chemical shift evolution schemes. To achieve a high resolution in the ¹³C _{α} dimension without causing splitting due to ¹³C-¹³C couplings, ¹³C _{β} -decoupling 180° pulses were used in the ¹³C _{α} chemical shift evolution schemes. The heteronuclear correlation spectra were recorded at 25°C using Bruker Avance III 800-MHz or 750-MHz spectrometers equipped with a cryogenic TCI probe. NMR data were processed and analyzed with NMR-Pipe⁷ and NMRFAM-SPARKY⁸ programs.

NMR experiments to determine ϕ_{ENS} potentials

Four stock solutions of 1) 60 mM aminomethyl-PROXYL, 20 mM Tris•acetate, 10 mM DMSO, and 5% D₂O at pH 7.5; 2) 50 mM carbamoyl-PROXYL, 20 mM Tris•acetate, 10 mM DMSO, and 5% D₂O at pH 7.5; 3) 60 mM aminomethyl-PROXYL, 10 mM K•HPO₄, 100 mM NaCl, and 5% D₂O at pH 7.4 and 4) 50 mM carboxy-PROXYL, 10 mM K•HPO₄, 100 mM NaCl, and 5% D₂O at pH 7.4 were prepared prior to preparation of the paramagnetic samples of proteins. The concentrations of aminomethyl-PROXYL, carboxy-PROXYL and carbamoyl-PROXYL were measured as previously described.⁹ These stock solutions were mixed with a protein solution in the same buffer so that the desired concentration (20 mM) of the paramagnetic cosolute was achieved in the final protein solution without altering the buffer. The paramagnetic samples of DERT30 contained 0.4 mM ¹³C/¹⁵N DERT30, 20 mM Tris•acetate, 100 mM NaCl, 10 mM DMSO, 5% D₂O, and 20 mM aminomethyl-PROXYL or carbamoyl-PROXYL. The paramagnetic samples for the full-length HMGB1 protein or the Δ30 variant contained 0.4 mM ¹⁵N-labeled protein, 10 mM potassium phosphate, 100 mM NaCl, and 5% D₂O at pH 7.4 with 20mM aminomethyl-PROXYL or carboxy-PROXYL, respectively.

A Bruker Avance III 800-MHz spectrometer equipped with a cryogenic TCI cryoprobe was used to measure solvent PRE rates Γ_2 for backbone ¹H nuclei as described previously.⁹⁻¹⁰ The sample temperature was 25°C. The effective near-surface electrostatic potential ϕ_{ENS} was determined from the PRE data using:¹¹

$$\phi_{ENS} = \frac{k_B T}{(z_b - z_a)e} \ln \left(\frac{\Gamma_{2,a}}{\Gamma_{2,b}} \right) \quad [s1],$$

where $\Gamma_{2,a}$ and $\Gamma_{2,b}$ are the solvent PRE rates for aminomethyl-PROXYL and carbamoyl-PROXYL/carboxy-PROXYL, respectively; z is the valance of electric charge ($z = 1$ for aminomethyl-PROXYL; $z = 0$ for carbamoyl-PROXYL; and $z = -1$ for carboxy-PROXYL); e is the elementary electric charge; k_B is the Boltzmann constant; and T is temperature. As previously described, the ϕ_{ENS} potentials were determined only for ¹H nuclei that satisfied the following criteria: $\Gamma_{2,a} > 3\sigma_a$; $\Gamma_{2,b} > 3\sigma_b$; and $\Gamma_{2,a} / \Gamma_{2,b} > 3\sigma_{ratio}$, where σ_a , σ_b and σ_{ratio} are the uncertainties in $\Gamma_{2,a}$, $\Gamma_{2,b}$, and $\Gamma_{2,a} / \Gamma_{2,b}$. The uncertainties in ϕ_{ENS} potentials were determined using:⁹

$$\sigma_\phi = \frac{k_B T}{|z_b - z_a|e} \sqrt{(\sigma_a / \Gamma_{2,a})^2 + (\sigma_b / \Gamma_{2,b})^2} \quad [s2].$$

MATLAB was used to calculate the ϕ_{ENS} potentials and their uncertainties.

Poisson-Boltzmann theory-based prediction for structure models

All model structures of the DERT30 peptide shown in Figure S5 were generated using the Xplor-NIH software.¹² The α -helix structure was generated through simulated annealing using backbone torsion angle restraints of $\Phi = -60 \pm 10^\circ$ and $\Psi = -50 \pm 10^\circ$ (through the ‘CDIH’ potential) and backbone hydrogen bond geometry restraints (through the ‘HBDA’ and ‘HBDB’ potentials¹³⁻¹⁴) under the influence of the conformational database potential¹⁵. The extended β -strand was generated through simulated annealing using torsion angle restraints of $\Phi = -140 \pm 1^\circ$ and $\Psi = 130$

$\pm 1^\circ$ for backbone together under the influence of conformational database potentials. A total of 200 random coil structures were generated through simulated annealing with a restraint on the radius of gyration (R_{gyr})¹⁶ under the influence of the conformational database potential. An ensemble of 100 structures was generated using the setting of $R_{gyr} = 15.0 \text{ \AA}$, which is based on the empirical equation of Kohn et al. for random coils.¹⁷ Another ensemble of 100 structures was generated using $R_{gyr} = 20.0 \text{ \AA}$ based on speculation that electrostatic repulsion between negatively charged side chains may significantly increase the radius of gyration. For each generated structure model, a PQR-format¹⁸ file containing the coordinates, the charge and the radius for each atom was created using a PDB2PQR program¹⁹. The charge and radius parameters of the AMBER force field were used. Nonlinear Poisson-Boltzmann equation-based electrostatic potentials at the ionic strength of 130 mM were computed for 3D lattice space with a grid interval of $0.5 \text{ \AA}$ using the APBS software²⁰. The effective near-surface electrostatic potential was predicted using:⁹⁻¹¹

$$\phi_{ENS}^{PB} = \frac{k_B T}{(z_b - z_a)e} \ln \left(\frac{\sum_i \rho_i r_i^{-6} \exp[-\frac{z_a e \phi_i}{k_B T}]}{\sum_i \rho_i r_i^{-6} \exp[-\frac{z_b e \phi_i}{k_B T}]} \right) \quad [s3],$$

in which ϕ_i is the Poisson-Boltzmann electrostatic potential at the grid point i ; r_i is the distance from the ^1H nucleus to the grid point i ; and ρ_i is a factor that represents the accessibility of the grid point i and is either 1 (accessible) or 0 (inaccessible).

²³Na NMR-based quantification of counterions condensed around DERT30

Ion-counting methods with buffer equilibration were originally developed by Herschlag and coworkers for quantification of counterions around nuclei acids using atomic emission spectroscopy²¹⁻²³ and inductively coupled plasma mass spectrometry²⁴⁻²⁶. Recently, our group adopted the ion-counting methods for NMR spectroscopy and applied to $^7\text{Li}^+$, $^{23}\text{Na}^+$, $^{133}\text{Cs}^+$, $^{15}\text{NH}_4^+$, and ^{13}C acetate ions around biomolecules.²⁷⁻³⁰ We used ^{23}Na NMR in the current study. To obtain the data on Na^+ ions shown in Figure 2A, the DERT30 solutions were equilibrated with a buffer at pH 7.5 containing 20 mM Na^+ . This buffer, which contains Na^+ as the sole cation, was prepared using 20 mM NaOH titrated with HEPES to adjust pH to 7.5. This buffer preparation method was chosen to avoid any additional cations that would interfere with our NMR-based ion-counting experiments. The buffer equilibration was conducted using Amicon Ultra-4 centrifugal filters (molecular weight cutoff at 3 kDa) until the overall dilution factor exceeds 10,000. ^{23}Na NMR experiments were conducted at 25°C using a Bruker Avance III spectrometer with an Oxford 17.6-T magnet, where ^1H and ^{23}Na resonance frequencies are 750 and 198 MHz, respectively, using a DiffBB probe of the broadband observation (BBO) configuration. In each NMR experiment, a coaxial NMR tube was used. The outer tube was a standard 5-mm NMR tube (Norell, Part# S-5-600-7) and the inner tube was a coaxial 2-mm stem insert (Norell, Part# NI5CCI-B). The outer tube contained a 380- μl DERT30 solution, whereas the inner tube contained a reference solution. The reference solution for the ^{23}Na NMR experiments was 300 mM NaOH, 20% H_2SO_4 , and 80% D_2O . The reference solution was designed to produce a $^{23}\text{Na}^+$ NMR signal at a position different from the position of the NMR signal from the cations in the DERT30 solution. ^{23}Na chemical shifts were referenced to the ^{23}Na signal from the reference solution in the coaxial inner tube (-1.857

ppm with respect to 0.1 M NaCl in D₂O). The intensity ratio for the two signals was used to quantify cations in the solutions in the outer tube (see below). Another advantage of the use of coaxial tubes is that the annular geometry of the outer-tube liquid suppresses convection that may adversely impact diffusion measurements.³¹

The total concentration of Na⁺ ions ($[\text{Na}^+]_{\text{total}}$), including those in the free and DERT30-bound states, in each DERT30 solution after buffer equilibration was determined from the ratio of the integral of the ²³Na NMR signal from the DERT30 solution to the integral of that from the reference solution ($r = I / I_{\text{ref}}$). The Na⁺ concentration in each sample was calculated as $[\text{Na}^+]_{\text{buffer}} \times r_{\text{sample}} / r_{\text{buffer}}$, where $[\text{Na}^+]_{\text{buffer}}$ is the Na⁺ concentration in the buffer (i.e., 20 mM) used for the equilibration process; r_{sample} is the integral ratio for the DERT30 solution sample; and r_{buffer} is the integral ratio for the buffer.²⁸ Because variations in thickness of the coaxial stem-insert glass wall can cause variation in the reference intensity, an identical coaxial stem-insert containing the reference solution was used for all samples of the same series. The dependence of $[\text{M}^+]_{\text{total}}$ on the DERT30 concentration (C_D) was analyzed using:²⁸

$$[\text{Na}^+]_{\text{total}} = [\text{Na}^+]_{\text{buffer}} + aC_D \quad [\text{s4}].$$

In Eq. s4, the parameter a corresponds to the ion excess ΔN_{cation} in the main text and was determined as the slope in the linear regression for the C_D -dependent $[\text{Na}^+]_{\text{total}}$ data (Figure 2A).

²³Na diffusion experiments

We conducted ²³Na diffusion NMR experiments as described in our previous study on Na⁺ ions around DNA. To obtain the Na⁺ diffusion data shown in Figure 2B, the stimulated echo with bipolar pulsed-gradient pair-longitudinal Eddy-current delay (BPP-LED) pulse sequence³² was applied to ²³Na nuclei using Bruker's standard pulse program ('stebpgp1s'). In the ²³Na diffusion NMR experiment, the magnetic field gradients were varied with 11 different strengths (i.e., 13.6, 38.0, 62.4, 86.9, 111.3, 135.7, 160.1, 184.6, 209.0, 233.5, and 257.9 gauss/cm). The self-diffusion coefficient of liquid N,N-dimethylformamide at 25°C was used to calibrate the pulsed field gradient (PFG) strengths.³³ Each PFG in the BPP schemes was 1.0 ms. The delay Δ between the beginnings of the first and second BPP schemes was 20 ms in the ²³Na diffusion measurements. To determine the apparent diffusion coefficient D for each sample, the following equation was used for nonlinear least-squares fitting for the signal intensity I :³⁴

$$I = I_0 \exp \left[-D\gamma^2 g^2 \delta^2 \left(\Delta - \frac{\delta}{3} - \frac{\tau}{2} \right) \right] \quad [\text{s5}],$$

in which γ , the ²³Na nuclear gyromagnetic ratio; g , the PFG strength; δ , the total length of a pair of bipolar PFGs; and τ , the time between two gradients in each spin echo. The ²³Na BPP-LED data were processed and analyzed using Bruker TopSpin software.

The apparent diffusion coefficient (D_{app}) of Na⁺ ions was measured for the solutions of DERT30 at varied concentrations. The following equation for fast exchange systems²⁷ was used to analyze the D_{app} data:²⁸

$$D_{\text{app}} = p_f D_f + p_b D_b = D_f + (D_b - D_f) aC_D / ([\text{Na}^+]_{\text{buffer}} + aC_D) \quad [\text{s6}],$$

where D_f and D_b are the diffusion coefficients for cations in the free state and those within the ion atmosphere, respectively. The parameter a in Eq. s6 is identical to that in Eq. s4 and was experimentally determined. The diffusion coefficient D_b for cations within the ion atmosphere was determined from the D_{app} coefficients through nonlinear least-squares fitting with Eq. s6 using the MATLAB software.

Measurement of binding affinity for four-way junction DNA

The binding affinity of the HMGB1 $\Delta 30$ variant protein for the four-way junction DNA J1 was measured using fluorescence-labeled J1 in which one of the four strands was labeled with fluorescein amidite (FAM). FAM fluorescence anisotropy was measured with an ISS PC-1 spectrofluorometer using an excitation wavelength of 490 nm and an emission wavelength of 521 nm at 25°C. The binding assays for FAM-labeled four-way junction was conducted in a buffer of 10mM potassium phosphate (pH 7.4) and 150 mM NaCl. The concentration of FAM-labeled DNA was 4 nM. The dissociation constant K_d was determined from the anisotropy data using MATLAB (MathWorks, Inc), as previously described³⁵. The binding affinity of full-length HMGB1 for the four-way junction DNA J1 was previously measured under the same conditions. The measurements of each dissociation constant K_d were triplicated.

Stopped-flow kinetic experiments on dissociation of protein from DNA

The dissociation kinetics were measured for the complexes with cisplatin-modified 20-bp DNA at 25°C using an Applied Photophysics SX20-LED stopped-flow spectrofluorometer. A polarized LED light with maximum intensity at 470 nm was used for excitation of the FAM fluorophore. The fluorescence anisotropy was measured in a real-time manner using two emission channels placed in a T-format configuration with a polarizer and a long-pass filter with a cutoff at 515 nm for each. For each kinetic measurement, the following two solutions were rapidly mixed in a 1:1 volume ratio (80 μ l each) by the stopped-flow device: 1) a solution of a complex formed with 50 nM protein (either full-length HMGB1 or $\Delta 30$) and 10 nM FAM-labeled 20-bp DNA containing a cisplatin modification; and 2) a solution of varied concentrations (500-2000 nM) of the 20-bp cisplatin-modified DNA with no FAM label. Both solutions were in a buffer containing 10 mM potassium phosphate (pH 7.4) and 100 mM NaCl. Immediately after the flow for mixing had been stopped, the time course data of fluorescence anisotropy were collected for a period of up to 30 s with time intervals ranging from 0.001-0.01 s. For each condition, the measurement was replicated 8-10 times. The obtained anisotropy decay curves were fitted with a single-exponential function. The apparent dissociation rate constants obtained by the fitting were plotted against the concentration of unlabeled competitor DNA and fitted with a linear function. The slope corresponds to the rate constant for intersegment transfer.

2. Bloch-McConnell equation-based simulations of NMR experiments on ^{15}N DERT30 competing with DNA J1 for the $\Delta 30$ variant of HMGB1

The system of ^{15}N -labeled DERT30 in the presence of the $\Delta 30$ variant of HMGB1 (unlabeled) and DNA J1 (unlabeled) can be represented by the following scheme:



where the molecules A and B compete for the ligand L, corresponding to DERT30, J1, and the $\Delta 30$, respectively. The equilibrium concentrations $[\text{A}]$, $[\text{B}]$, $[\text{L}]$, $[\text{AL}]$, and $[\text{BL}]$ can be calculated from the total concentrations (A_{tot} , B_{tot} , and L_{tot}) and the dissociation constants ($K_{d,A}$ and $K_{d,B}$) by solving the following simultaneous equations.

$$[\text{A}] + [\text{AL}] = A_{\text{tot}} \quad [\text{s9}]$$

$$[\text{B}] + [\text{BL}] = B_{\text{tot}} \quad [\text{s10}]$$

$$[\text{L}] + [\text{AL}] + [\text{BL}] = L_{\text{tot}} \quad [\text{s11}]$$

$$[\text{A}][\text{L}]/[\text{AL}] = K_{d,A} = k_{\text{off},A}/k_{\text{on},A} \quad [\text{s12}]$$

$$[\text{B}][\text{L}]/[\text{BL}] = K_{d,B} = k_{\text{off},B}/k_{\text{on},B} \quad [\text{s13}]$$

In Eqs. s12 and s13, k_{on} and k_{off} represent a second-order association rate constant and a first-order dissociation rate constant, respectively. The behavior of transverse magnetization of A can be described by the following Bloch-McConnell equation:

$$\frac{d}{dt} \mathbf{m} = (\mathbf{K} - \mathbf{R} + i\mathbf{W})\mathbf{m} \quad [\text{s14}],$$

where $\mathbf{m}$ is a two-dimensional column vector with each element representing magnetization in a form of $m_x + im_y$ for A and AL (i.e., free ^{15}N DERT30 and ligand-bound ^{15}N DERT30); $\mathbf{K}$ is a kinetic matrix; $\mathbf{R}$ is a relaxation matrix; and $\mathbf{W}$ is a chemical shift matrix, and i is the imaginary number. The matrices are given by:

$$\mathbf{K} = \begin{bmatrix} -k_{\text{on},A}[\text{L}] & k_{\text{off},A} \\ k_{\text{on},A}[\text{L}] & -k_{\text{off},A} \end{bmatrix} \quad [\text{s15}]$$

$$\mathbf{R} = \begin{bmatrix} R_{2,A} & 0 \\ 0 & R_{2,AL} \end{bmatrix} \quad [\text{s16}]$$

$$\mathbf{W} = \begin{bmatrix} \Omega_A & 0 \\ 0 & \Omega_{AL} \end{bmatrix} \quad [\text{s17}]$$

The solution of Eq. s14 is given by:

$$\mathbf{m}(t) = \exp[(\mathbf{K} - \mathbf{R} + i\mathbf{W})t]\mathbf{m}(0) \quad [\text{s18}]$$

Fourier transformation of the sum of $\mathbf{m}(t)$ elements into a frequency domain gives an NMR spectrum.

Using Eq. s9-s18, we simulated ^{15}N NMR spectra for ^{15}N DERT30 as a function of the concentration of DNA J1 (which corresponds to B_{tot} in Eq. s10). The simulated spectra are shown in Figure S10.

3. Rate equation-based simulations of kinetic experiments shown in Figure 4B

System involving dissociation and association processes

In the stopped-flow experiments shown in Figure 4B, the system involves a protein (P), fluorescence-labeled DNA (D_a), and unlabeled DNA (D_b). The dissociation kinetics was analyzed by monitoring fluorescence immediately after a solution of P and D_a , in which D_a is predominantly in a bound state (PD_a), was mixed with a high-concentration solution of D_b (Figure S11). The protein transfers from D_a to D_b through dissociation ($PD_a \rightarrow P + D_a$) and association ($P + D_b \rightarrow PD_b$). The rate equations for this system are given as follows:

$$\frac{d}{dt}[P] = -k_{on}[P]([D_a] + [D_b]) + k_{off}([PD_a] + [PD_b]) \quad [s19]$$

$$\frac{d}{dt}[D_a] = -k_{on}[P][D_a] + k_{off}[PD_a] \quad [s20]$$

$$\frac{d}{dt}[D_b] = -k_{on}[P][D_b] + k_{off}[PD_b] \quad [s21]$$

$$\frac{d}{dt}[PD_a] = k_{on}[P][D_a] - k_{off}[PD_a] \quad [s22]$$

$$\frac{d}{dt}[PD_b] = k_{on}[P][D_b] - k_{off}[PD_b] \quad [s23]$$

An approximate analytical expression is useful to illustrate the meaning of the apparent rate constant from the stopped-flow experiment. Under the condition of $[D_a]_{total} \ll [P]_{total} \ll [D_b]_{total}$, which is the case in our experiments for Figure 4B, the molecular processes involving $[P]$, $[D_b]$, and $[PD_b]$ reach quasi-equilibrium before the system reaches the true equilibrium for all species. Using $k' = k_{on}[P]$, which is approximately constant due to this quasi-equilibrium, Eqs. s20 and s22 can be approximated with:

$$\frac{d}{dt}[D_a] = -k'[D_a] + k_{off}[PD_a] \quad [s20']$$

$$\frac{d}{dt}[PD_a] = k'[D_a] - k_{off}[PD_a] \quad [s22']$$

This approximation leads to:

$$[D_a](t) = \frac{D_{a,tot}k_{off}}{k_{off}+k'} - \left(\frac{D_{a,tot}k_{off}}{k_{off}+k'} - [D_a](0) \right) \exp[-(k_{off} + k')t] \quad [s24]$$

$$[PD_a](t) = \frac{D_{a,tot}k'}{k_{off}+k'} + \left(\frac{D_{a,tot}k_{off}}{k_{off}+k'} - [D_a](0) \right) \exp[-(k_{off} + k')t] \quad [s25],$$

where $D_{a,tot}$ is the total concentration of D_a (i.e., $[D_a] + [PD_a]$). Since Eqs. s24 and s25 share the same exponential term, the time course of the ensemble average of fluorescence $\langle F(t) \rangle$ from D_a and PD_a in the stopped-flow fluorescence experiment will be in a form of:

$$\langle F(t) \rangle = A + B \exp[-k_{off}^{app}t] \quad [s26],$$

where the apparent rate constant k_{off}^{app} is given by:

$$k_{off}^{app} = k_{off} + k' = k_{off} \left(1 + P_q/K_d \right) = k_{off} \left(1 + \frac{P_{tot}}{K_d + D_{b,tot}} \right) \quad [s27].$$

In Eq. s27, P_{tot} is the total protein concentration; $D_{b,tot}$ is the total concentration of D_b ; and P_q is $[P]$ at the quasi-equilibrium and is given by $P_q = P_{tot}K_d/(K_d + D_{b,tot})$. When the second term of Eq. s27 is not negligible, k_{off}^{app} will decrease towards k_{off} upon an increase in $D_{b,tot}$ (see Figure S12A).

System involving dissociation, association, and intersegment transfer processes

Some proteins can directly transfer from PD_a to PD_b through a process called intersegment transfer.³⁶⁻³⁸ In this process, a protein molecule transiently bridges two DNA molecules and transfers from one DNA to another without going through the intermediary of the free protein. Phenomenologically, intersegment transfer can be regarded as a second-order process involving a collision between a protein-DNA complex and free DNA.^{37,39} When this additional second-order process is present, the rate equations Eq. s19-s23 should be modified as follows.

$$\frac{d}{dt}[P] = -k_{on}[P]([D_a] + [D_b]) + k_{off}([PD_a] + [PD_b]) \quad [s28]$$

$$\frac{d}{dt}[D_a] = -(k_{on}[P] + k_{it}[PD_b])[D_a] + (k_{off} + k_{it}[D_b])[PD_a] \quad [s29]$$

$$\frac{d}{dt}[D_b] = -(k_{on}[P] + k_{it}[PD_a])[D_b] + (k_{off} + k_{it}[D_a])[PD_b] \quad [s30]$$

$$\frac{d}{dt}[PD_a] = (k_{on}[P] + k_{it}[PD_b])[D_a] - (k_{off} + k_{it}[D_b])[PD_a] \quad [s31]$$

$$\frac{d}{dt}[PD_b] = (k_{on}[P] + k_{it}[PD_a])[D_b] - (k_{off} + k_{it}[D_a])[PD_b] \quad [s32],$$

where k_{it} is the second-order rate constant for intersegment transfer. As described above, under the condition of $[D_a]_{total} \ll [P]_{total} \ll [D_b]_{total}$, the molecular processes involving $[P]$, $[D_b]$, and $[PD_b]$ reach quasi-equilibrium before the system reaches the true equilibrium for all species. Thus, pseudo-first order approximation is applicable to Eqs. s29 and s31. As shown for Eqs. s20 and s22, the pseudo-first order approximation leads to an exponential function of $\langle F(t) \rangle$ in the form of Eq. s26, where the apparent rate constant k_{off}^{app} is given by:

$$k_{off}^{app} = k_{off} \left(1 + \frac{P_{tot}}{K_d + D_{b,tot}} \right) + k_{it}D_{b,tot} \quad [s33].$$

When $k_{off} \ll k_{it}D_{b,tot}$, the apparent rate constant k_{off}^{app} becomes a linear increasing function of $D_{b,tot}$ as seen for the data on the $\Delta 30$ variant of HMGB1 in Figure 4B (see also Figure S12B).

Table S1. D/E repeats of 10 or more consecutive aspartate (D) or glutamate (E) residues in human proteins. DNA-binding or RNA-binding proteins (according to Gene Ontology annotations) are indicated in bold. The information was obtained from the UniProt database (<https://www.uniprot.org/>).

Proteins	D/E repeats (residue numbers)	
ABCC9	951	EDEDEEEEEEEEEDEDD 965
ACIN1	270	EEEEEEEEEEEEEDDEEEE 287
ADRA2B	298	EEEEEEEEEEEE 309
AEBP2	37	EEEEEEEEEEEE 48
AEBP2	95	EDEDEEEDDEEEEEE 109
Afadin	1577	DEDEEEEEEDDD 1587
ALMS1	13	EEEEEEEEEEEEEEEE 28
AMER1	376	DDDEEEEEEEEE 387
ANAPC15	63	DDEEEEDDEDED 75
ANKRD17	117	EEEEDDDEEEEE 128
ANKRD40	96	EEEDDDDDDD 106
Anoctamin-8	582	EEDEDEEEEEDEEEEEDEEE 601
ANP32A	168	DDEEEDDEEEE 178
ANP32A	187	EDEEEDDEEEE 197
ANP32B	175	EDEEEDDED 184
ANP32B	219	DEEDEDDEEEEE 233
ANP32E	185	EEEEEEEEDEDEDEDEDE 204
APBB1	158	EEEEEDDDDEEEED 172
APLP2	215	EEEEEEEEEEEEDEED 232
ARID1B	1726	DDDEEEDDEEED 1738
ARID3A	148	EDEEEEEDEE 157
ARID4B	279	EEEEEEEDDE 288
ARID4B	543	EEEEEEEEEEEEDEDDDD 559
ARIH1	25	EEEEEDDDDE 34
ARIH2	22	EEEEEEEEEDD 31
ARMC9	583	DDDEEDDEED 593
ARMH4	614	EDEDEEEDDEEEEEDEEED 637
ARX	232	EDEEEDDEEEE 242
ASCC2	658	EEDDDDEEDD 667
Asporin	38	DDDDDDDDDDDDDD 51
ATAD2	258	EDEDEDDDDDDDDDDDDDEDEDEED 285
Atherin	329	EEEEDDDEDEDEEDD 343
ATRX	1450	EEEEEEEEEEEEEEEE 1466
BAHCC1	1876	EEEEEEEEEEED 1887
BAZ1B	1260	DEEEEEEEEEEEED 1274
BAZ2B	609	EDEEEDDEEEDDEDEDE 627
BBX	41	EEEEEEDEED 51
BCL11A	484	DEEEDDEEEEEEEEEEEEE 504
BCL11B	531	EEDEEEEEEEEE 543
BOD1L1	2987	DEEEEEEEDE 2997
BRD2	492	EEEEEEEDDEEEEE 506
BRG1	663	EEEEEEEEEE 672
CACNA1D	827	EEEEEEEEDE 836
CACNA1F	809	EEEEEEEEEEEEEEEE 825
CASQ2	373	EDDDEDDDDDD 383
CASZ1	1690	EDEDEDDDEDDDDDEDDDDDED 1714
CCDC136	15	EEEEEEEEEEEE 26
CCDC28B	141	EEEDDEEED 150
CDK11a	292	EEEEEEEEEEEE 304
CDK11a	312	EEEEEEEEEEEE 324
CDK11b	304	EEEEEEEEEEEE 316
CDK11b	324	EEEEEEEEEEEE 336
CELSR2	2743	EEEEEEEEEE 2752
CENP-B	406	EEEEEEEEEEEE 419

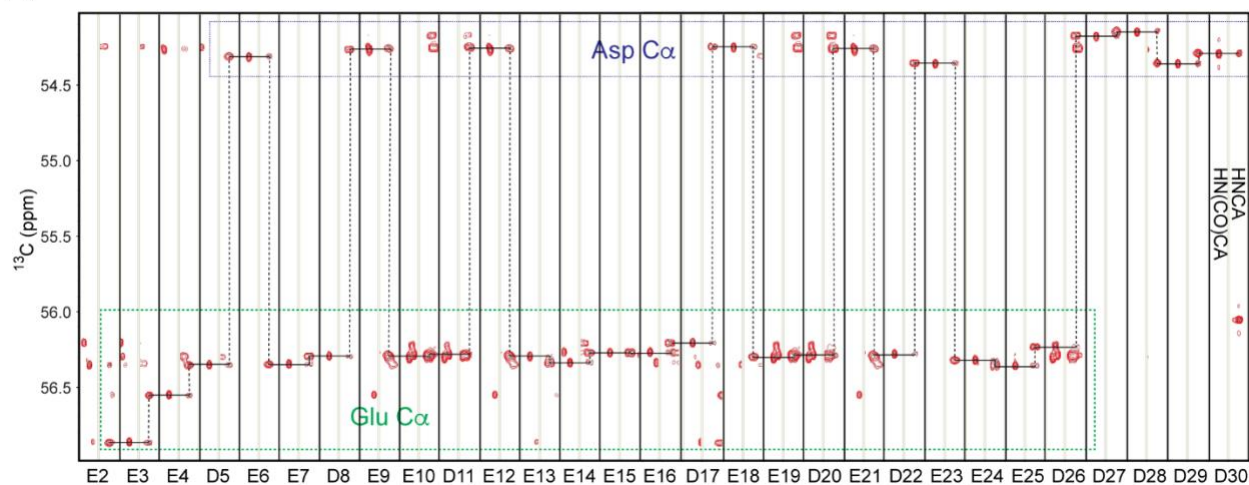
CENP-B	515	EEEDDEEDEDDEDDDDDEED	535
CERS3	346	EEEEEEEEEE	355
CFAP43	956	EEDDEEEEEED	966
CHD4	134	EEEEEDDDDD	144
CHD8	2537	EEDDDEEEDDDD	2549
CHIC1	42	DDDEEDEDDEEEEEEEEEEEEEEE	68
CIZ1	743	DEEEEDDEDEEE	755
CLIP3	16	EEEEEEEEDE	25
CLSTN1	925	EEEEEEEEEE	935
CNGB1	355	EDEEEEEEEEEEEEE	371
CNKS2	877	EEEEEEEEEE	886
CNPY4	220	EEEEEEEEEE	229
CSPG5	276	DEEEEEEDD	285
CSRNP3	370	DEEEEEEEEEEEEEDDDD	388
Cullin-9	1671	EEEEEEEEEE	1681
Cullin-9	2463	EEEEEDDED	2472
CWC22	423	EDEEEEEEE	432
DAXX	444	DEEEEEEEEEEE	457
DAXX	475	EDDEEEDDEEE	485
DCAF1	1410	EEEDDDEDDDD	1420
DCAF8	100	EEEEEEEEEEEE	112
DCAF8L2	126	EEEEEEEEEEEEEEEEEEEEEE	147
DEK	33	EEEEEDEDDEEEEEEE	47
Dematin	216	EEEEEEEDDD	225
Dp-2	432	DEDEDDEED	441
EHMT2	300	EEEEEEEEEEEEEEEEEEEEEEDEE	326
eIF3D	530	DEEEEEEEEEEEEEEEEE	547
eIF5B	532	EEEEEEEEEEEEEEDEE	546
EP400	426	EEEEEEEEEEEE	437
Ephexin-1	216	EEEEEEEEEE	226
ERCC6	378	EEEEEEEDDE	387
ERCC6-PGBD3	378	EEEEEEEDDE	387
ESF1	273	EDDEEDEDDEEDED	289
FAM171A1	858	EEEEDDDDDD	867
FAM193A	905	EEEEEEEEEE	915
FAM43B	265	EDEEEEEEDD	274
FAM9A	262	EEEEEEEEEEEE	275
FBXO3	424	EEEEEEEEEEEDDD	438
FGD1	340	EEEDDEEEEE	350
FKBP8	36	EEEEEEEEEE	46
FOXD1	26	EDEEEDDEEDDE	38
FOXO3	59	EEEDDEDDED	68
FOXO3B	144	EEEDDEDDED	153
GOLGA2	704	EEEEEEEEEEEE	715
GRWD1	123	DEEEEEDEEDEEE	136
GSE1	1102	EEEEEDDED	1111
GZF1	298	EEEEEEEEEEDE	309
HAP1	260	DEEDEEEEEEE	272
HAX1	30	DEDDDEEEEE	40
HDGF	213	EEEEEEDEEE	223
HINFP	49	EEEEEEEDD	58
HIVEP3	1860	DEDEDEEEE	1869
HMGB1	186	EEEEDEEDEDDEEDEDDEEEDDDDE	215
HMGB1P1	186	EEEEDEEDEDDEEDEDDEEEDDDDE	211
HMGB2	188	EDEEEEEDEEDEDDEE	209
HMGB3	181	EEEEEEEEEEEEEEEEDE	200
hnRNP U	83	EEEEEEEEEE	94
hnRNP UL2	74	DEEEDDEEEDDEE	87
Homez	514	EEEEEEEEEE	523
Homez	526	EDDEEEEEEEEDDDDDDD	545
Hox-A7	217	DEEDDEEEDDEE	230
HRC	193	EEEEEEEEEE	204
HRC	244	EEDDDDDDDDDDDDDDD	261

HRC	541	EEEEEEEEDEE	550
HUWE1	2433	DEEEEEEEEEDEDD	2445
IFNLR1	324	EDEEEEEDEED	333
IGSF3	1014	EEEEEDDDDDDD	1026
IL27	164	EEEEEEEEEEEEEE	176
Importin-4	814	DEEEEEEDDD	823
Integrin α E	189	EEEEEEEEEE	198
IRX3	211	EEEEEEEEDEED	221
Kanadaplin	648	EEEEEEEEEEEE	658
KANK1	1033	EEEEEEEEDEED	1042
KAT6B	1070	EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	1104
KAT6B	1351	EEEEEEEEEEEEEEEEEEEE	1368
KCNA4	123	EEEEEEEEEEEEEEEE	137
KCNN1	67	DDDEDDEEDE	76
KDM2A	856	EEEEEEEEEEEEEDD	868
KIF21A	611	EDEEEEEEEEEEDD	623
KRI1	250	EEEEEEEEDEEE	261
LAS1L	589	EEEEDEDEDDEDEED	604
LDOL1	132	DDDDDDDEEEEDD	145
Leiomodin-3	144	DEEEEEEDDDDDDE	158
LMTK3	1279	EDEDEEEEEDEE	1290
MAPK8IP1	107	EDDEEDDDEE	116
MAPK8IP2	88	EEEDDEEEEEEEEEEE	103
MAPK8IP2	469	EEDEEDDEEEED	481
MBD3	268	EDDDEEEEEEEEE	281
Midasin	4781	DDDEEEEEDEED	4792
MIER1	83	EEEEEEEEEEEE	94
MNX1	357	EEDEDEDDED	366
mTERF4	340	DEDEDDDEED	350
mTERF4	372	DEDEDDDEEE	381
mTERF4	357	DEDDDDDEDDDE	367
MYO15B	1002	EEEEEEEEEEEE	1012
MYT1	258	EE	306
MYT1L	136	EEDEDDDED	145
MYT1L	150	EDEEEEEEEEEEEEEEE	167
N-Myc	264	DDDEDEEEEEEE	276
N-WASP	486	DEDEDEDDEED	496
NAP1L2	213	EEEEEEEEEDD	223
Nardilysin	150	EEEEEDDDDED	159
NBPF4	167	DEDEDEDED	176
NBPF5P	167	DEDEDEDED	176
NBPF6	167	DEDEDEDED	176
NBR1	703	EEEEDEEEEEDEE	716
NCBP3	344	EEEEEEEEEEEEEEED	360
Neurabin-2	411	EEDEDEDDED	420
NEUROD1	67	EEEEEEEEEDDD	78
NEUROD2	82	EEEEEEEEEE	91
Nischarin	637	EEEEEEEEEEED	648
Nischarin	672	EEEEEEEEDEE	682
NPM1	161	DEDDDDDEEDDDDEDDDDDD	180
NPM2	137	EEEEEEEEDEDED	150
NT5C2	549	DEDDDEEEEEEE	561
Nucleolin	154	EEDEEDDEDEDEDEDE	169
Nucleolin	185	EDDEDEDDEDDEDDDDDEEDD	205
Nucleolin	234	EDEDEEDDEDEDDEDDDDDEDEDDEDEEEEEEEEE	271
OS-9	414	EEEEDEDEDEDEDE	429
PAK3	187	EEEEEEEEEEDE	199
PATL2	35	EEEEEEEEDEED	45
PAXIP1	189	EEEEEEEEEEEE	200
PCGF6	91	EEEEEEEEED	100
PDE4C	666	EEEEEEEEEE	675
PDIA4	39	EDEEEEEEDDDEEDD	55
PELP1	87	DEEEEEEEEEEEEEEEEEED	909

PELP1	943	EEEEEEEEEEEEEEEE	956
PERCC1	34	EEEEEEEEEEEEEEEE	47
Peregrin	465	EEDEDEEEDE	474
PEX14	324	EDEDEEDDD	333
PHACTR1	395	EEEEEEEEDEDD	406
PHACTR2	453	DDEDEDEDED	462
PHF23	248	EEEEEEEEEEEEEEEE	261
PIAS4	477	EDEEEEEEEEEDEEE	492
PIK3R5	319	DDEEEEEEEEE	329
PLC β 3	556	EDDEEEEEEEEE	566
PLC δ 3	499	EEEEEDDEEEEE	511
PLEKHG5	712	EEEEEEEEEEEE	723
PLPPR3	441	EEEEEEEEEEEEEEEEEEEE	461
Podocan	600	EEEEEEEEEEEE	611
PODXL2	162	EEEEEEEEEEEE	172
PPARGC1B	430	EEEEEEEEEEEEEEEE	444
PPARGC1B	814	EEEEEDDEEED	824
PPM1F	101	EEEEEEEDDDEEE	113
PPM1G	302	EEDDEEEEE	311
PPP1R15A	351	EEEEDEEDED	361
PPP1R1B	119	EEDEEEEEEDDEEEEEED	136
PPP4R2	298	EEDEEEEEEEEE	310
PPP4R3A	808	DDDEDDDEDED	818
PPP4R3B	829	DDEEEEEEEEE	838
PPP6R1	621	DDDEEEEEDEE	631
PPP6R1	671	EDEEEEEEEEEDEE	685
PRDM10	134	EEDEDEDED	143
PRDM2	268	EEEEEEEEEDDEEEDDDDE	288
PRKCSH	313	EEEEEEEEEEEE	325
PTMS	63	EEDEEEEEEDDE	75
RAD21	534	EDDEEDED	543
RanGAP1	359	DDEDEEEEE	368
RanGAP1	377	EEEEEEEEEEEEEEEEEEEE	397
RBM10	113	EEEEEEEEEEEE	125
RBM19	714	EEEEEEEEEEEE	725
RBM28	234	DDDDDDDEED	244
Reticulon-4	32	EDEEEEEEEEEDEDED	48
RNF34	222	EDDDDDDEDDDEEE	237
RNGTT	195	EDDEDEDED	205
RRP12	1054	EEEEEEEEEEEE	1064
RSPH4A	567	EEEEEEEEDEE	576
RYR1	1873	EEEEEEEEEEEE	1883
SALL2	764	EEEEEEEEEEEEED	776
SCAF1	268	EEEEEEEEEEEEEEEEEEEE	288
SCAF1	1022	EEEEEEEEEEEEEEEE	1039
Sec24B	377	DDEEEEEDEE	387
SEC63	750	EEEEEEEEEDDD	760
SECISBP2L	989	EEEEDEEEEEED	1001
SEN3	76	EEEEEEEEEEEEDEEE	92
SET	246	EEDDDDEEE	256
SETD1A	977	DEEDDEEDED	989
SETD1B	1185	EEEEEEEE	1194
Shroom4	1132	EEEEEEEEEEEEEEEEEEEE	1151
SKIDA1	419	EEEEEEEE	428
SLC24A1	879	EEEEEEEEEEEE	890
SLC24A3	421	DEEEEEEDDDE	432
SLC4A3	135	DEEEEEEEEE	145
SMARCA2	1518	EEEEEEDEE	1527
SMYD5	393	EEEEEEEE	403
SNAPC5	86	EEEEEEEE	95
SNIP1	383	DDEDEEEEE	392
SNRK	446	EDEEDED	455
SNX21	67	EDDEDDEDDEE	79

SOX-11	223	DEDDDDDDDDDE	234
SOX-12	163	EDDDEDDDEE	172
SPRYD3	373	EEEEEEEEEEEE	384
SPT5	43	EDEEEEEEEEE	53
SPT5	55	DEEEEEEDDD	64
SPT6	32	EEEDDDEEEEE	43
STAC3	65	EEEEEEEEEEEE	76
TAF1	1827	EEEEEEEEEE	1836
TAF7L	333	DDDEDEDEDEDEDEDEDEDEDE	354
TAF9	249	EDDDDDDDDDDD	260
TAOK2	378	EEEEEEEEEEEEEEEE	392
Taperin	607	EEEEEEEEEEEEEEEE	621
TF3C-γ	94	EDDEEEEEEEEEEEEE	111
TF3C-ε	486	EDDEEEEEEEEEED	500
TIMELESS	666	EEEEEEEEEEEE	678
TMED8	240	DEDEEEEEEEEE	251
TPR	1966	EEDEEDDDDDDD	1978
TRANK1	1087	EEEEEEEEEEEEED	1099
TRIM26	421	DEEEEEEEEE	432
TRIM52	122	DEEEEEEEED	131
TSPOAP1	1262	EEEEEEEEEEEE	1274
TSPOAP1	1334	EEEEEEEEDEEEE	1346
TTBK1	733	EEEEEEEEDEEEEEDEEEEEEEEEEEEEEEEEEEEE	771
TTLL3	71	EDEDEDEDEE	80
TULP1	115	EDEEEEEDEDEDEE	131
TUT7	845	DEEEDDEEEEEEE	859
UBTF	678	EEDDEEDEDEDEDEDEDEDEDE	698
UBTF	725	EEDDEDEDDDEDDDEDED	742
URI1	299	DDDDDDDDDDDD	311
UTP25	84	EEEEEEEEEEEEED	97
VGLL3	64	EEDEEEEEEE	74
VIRMA	258	EEDEDEDED	267
VIRMA	276	EEEEDEEEE	285
VSIG10	463	EEEEEEEEEEEEED	475
WDR70	154	EEEEEEEEEE	164
WEE1	34	EEEEEEEEEE	43
Wiz	567	EEEEEEEEEEED	577
YTHDC1	230	EEEEEEEEEEEEEEEE	249
YY1	43	EEEEDDDDDED	53
ZBTB12	195	EEDEDEDED	204
ZBTB4	637	EEDEEEEEEEEEEEEEDEE	654
ZBTB47	277	DEEEDDEEEEEEEEE	294
ZBTB47	326	EEEEEEEEEE	335
ZBTB7C	140	EDDDDDDEDDDEDEEEEEEEEEEDDDDD	167
ZBTB9	212	EEEEEEEDDDDED	225
ZEB1	1062	DEEEEEEEEE	1071
ZFHX3	473	EEEEEEEEEEEEEEEEDE	489
ZFTA	188	EEEEEEEEEEEE	200
ZMYND15	116	EEEEEEDEEEE	126
ZNF316	135	EEEEEEEEDEDED	148
ZNF326	498	EEDEDEDEEEE	508
ZNF428	43	EEDEEEEEEEEE	54
ZNF526	232	EEEEEDDEEDEDDEE	247
ZNF777	457	DEEEEEEEEEDE	467
ZNF830	160	EDEEEEEEEEEE	170

A



B

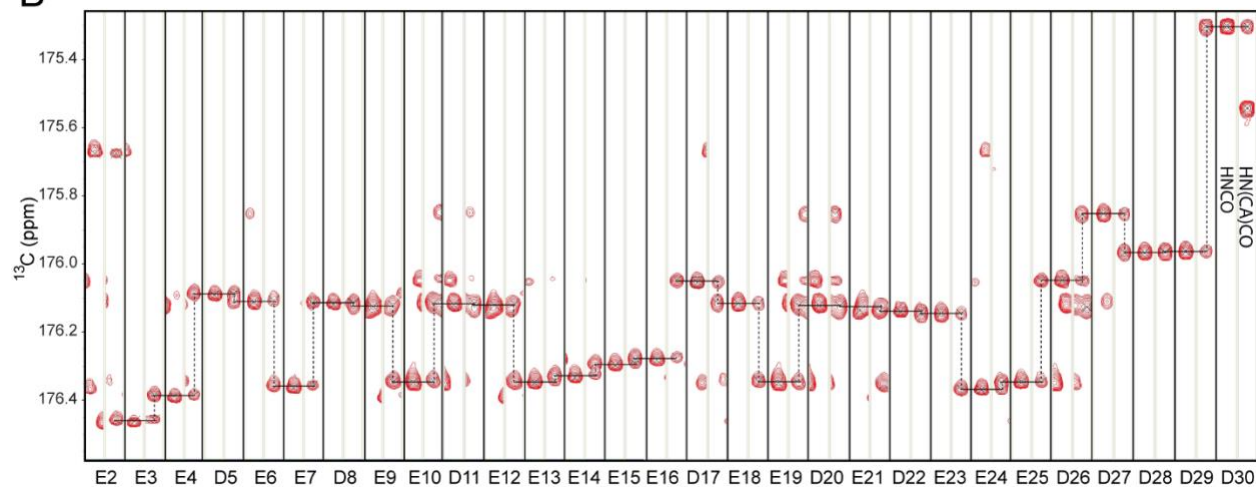


Figure S1. Sequential resonance assignment for the low-complexity sequence of DERT30. **(A)** $^{13}\text{C}_\alpha$ connectivity through the HNCA and HN(CO)CA spectra. **(B)** Backbone $^{13}\text{C}=\text{O}$ connectivity through the HN(CA)CO and HNCO spectra.

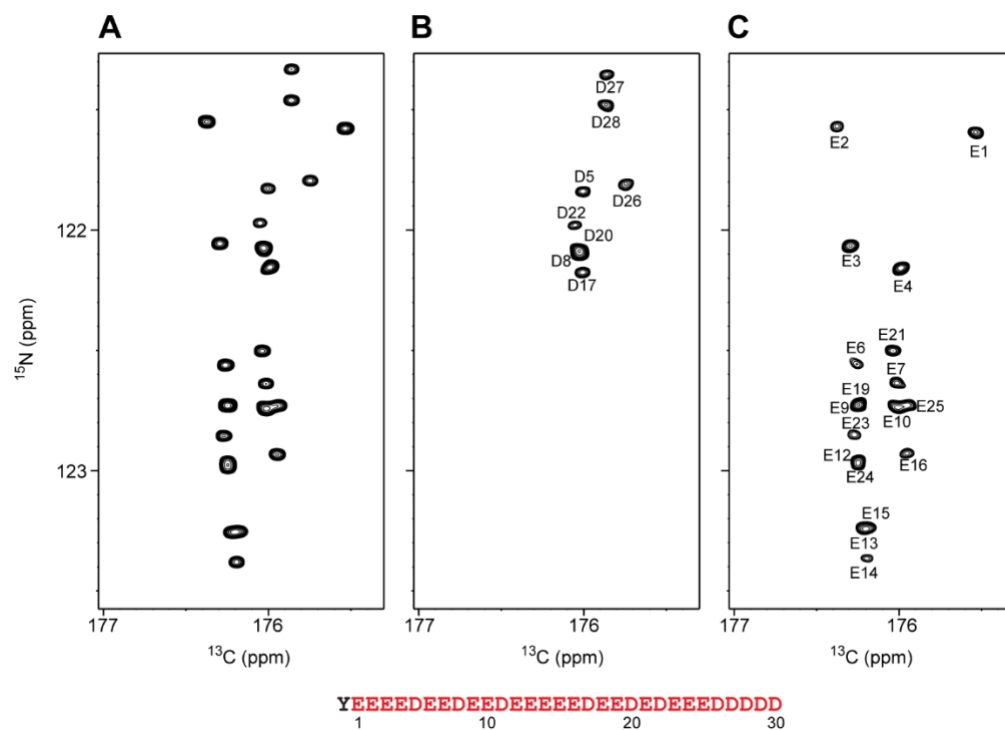


Figure S2. Direct ^{13}C -detected CON spectra recorded with and without an amino-acid type-selective filter⁵ for ^{13}C , ^{15}N -labeled DERT30. **(A)** CON spectrum recorded without any amino-acid type-selective filter. **(B)** Asp/Asn-selective CON spectrum. **(C)** Gln/Glu-selective CON spectrum. The annotations in Panels B and C indicate $^{13}\text{C}=\text{O}$ assignment. Note that the annotations in Figure 1B indicate ^{15}N resonance assignment.

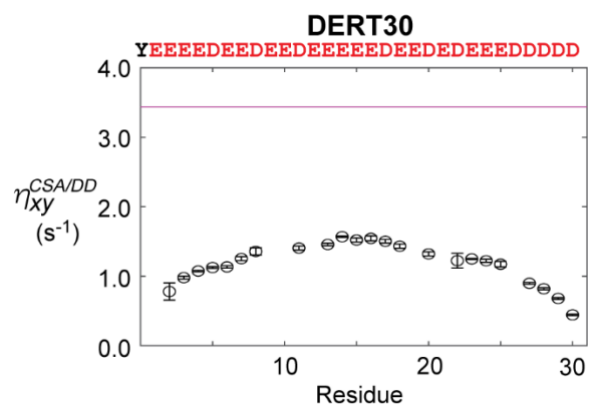


Figure S3. Transverse ¹⁵N CSA / ¹⁵N-¹H DD cross-correlation rates⁴⁰⁻⁴² measured for DERT30 NH groups. The purple line indicates the theoretical values for a rigid system with a rotational correlation time of 2 ns and an NH order parameter of 0.9. The observed values of the cross-correlation rates suggest the disordered nature of DERT30.

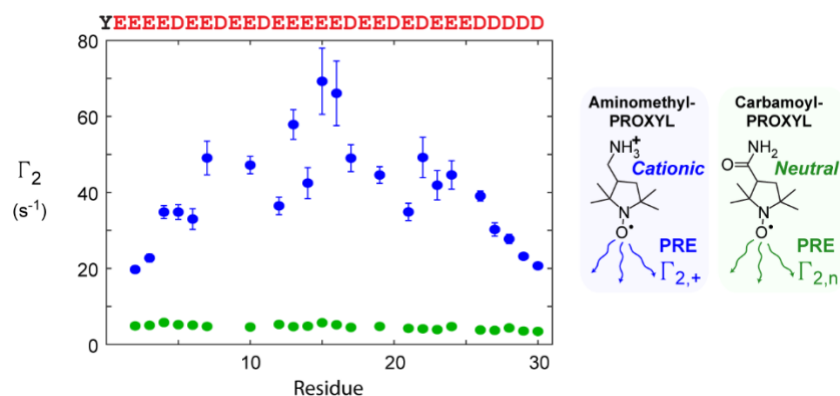


Figure S4. Solvent PRE data for $^1\text{H}_\alpha$ nuclei of DERT30. ^1H transverse PRE rates (Γ_2) measured for individual residues of DERT30 at 25°C and the ^1H frequency of 800 MHz in the presence of 20 mM aminomethyl-PROXYL or 20 mM carbamoyl-PROXYL. The solvent PRE data for aminomethyl-PROXYL and carbamoyl-PROXYL are shown in blue and green, respectively.

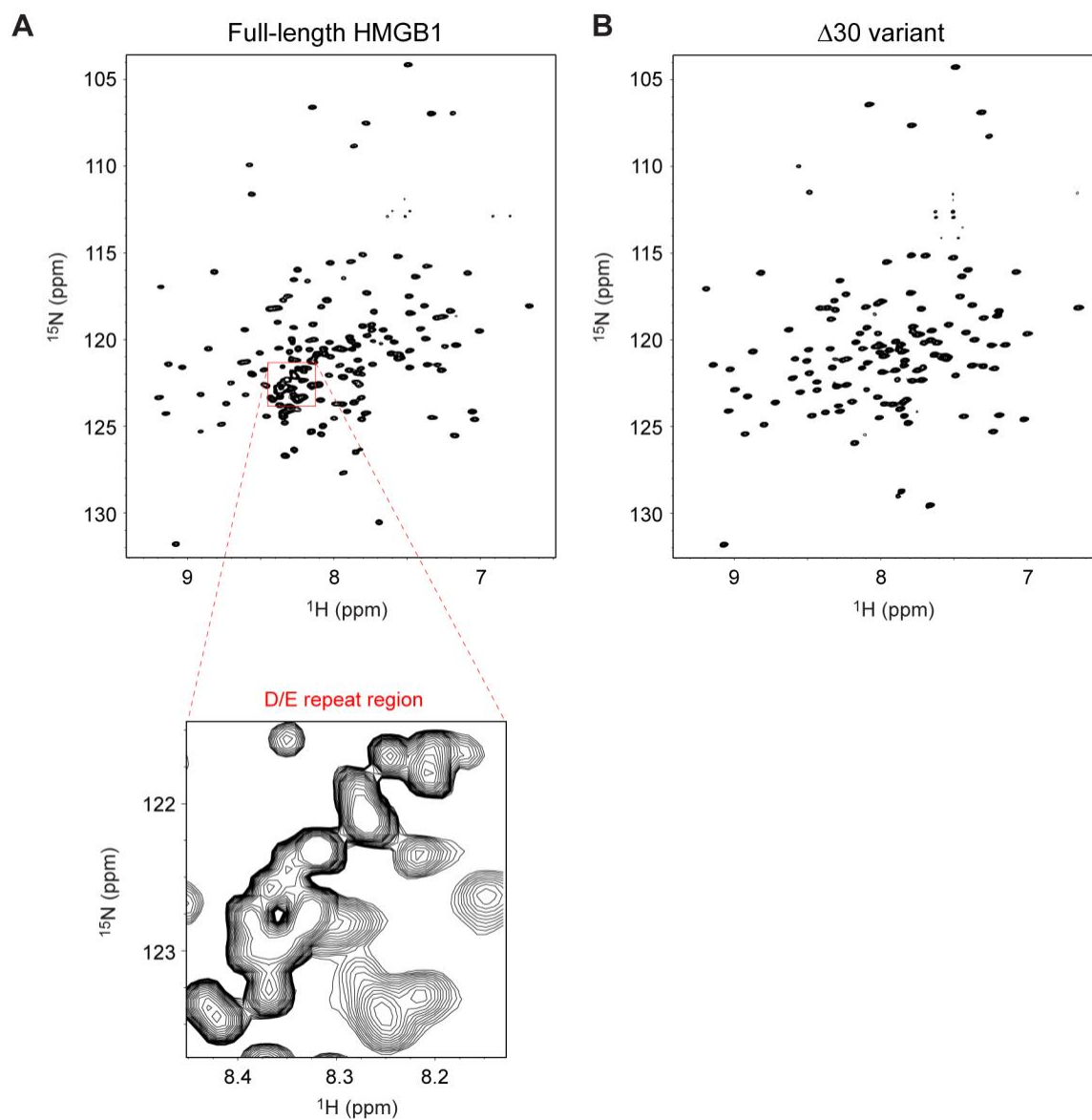


Figure S6. ^1H - ^{15}N TROSY spectra recorded at 25°C for the full-length HMGB1 protein and the $\Delta 30$ variant dissolved in a buffer of 10 mM potassium phosphate, 100 mM NaCl, and 5% D_2O at pH 7.4. These proteins are in the oxidized form with the C23-C45 disulfide bond.^{3,44}

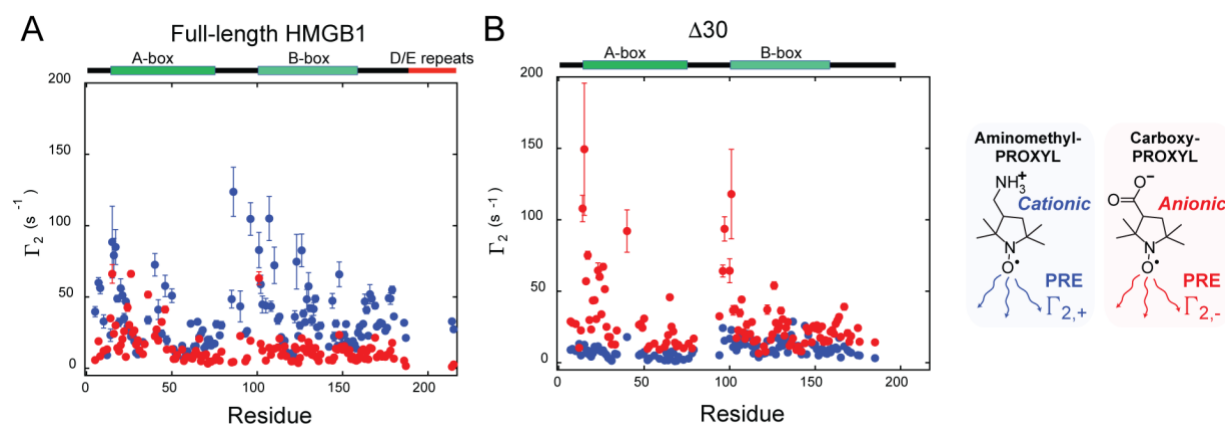


Figure S7. Solvent PRE rates Γ_2 measured for backbone ¹H_N nuclei of the full-length HMGB1 protein (Panel A) and the $\Delta 30$ variant lacking the D/E repeats (Panel B). The paramagnetic cosolute was either 20 mM aminomethyl-PROXYL (blue) or 20 mM carboxy-PROXYL (red).

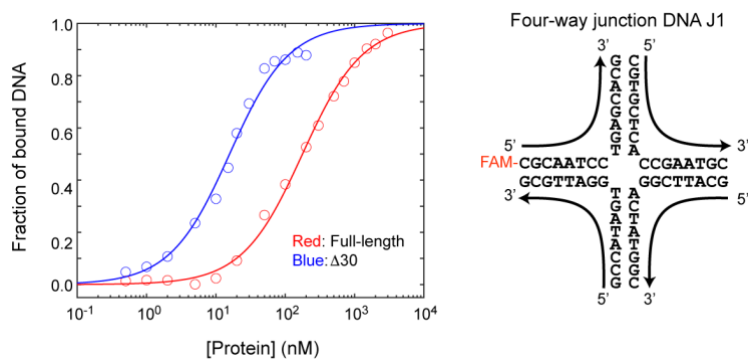


Figure S9. Fluorescence anisotropy-based binding assay data used to determine the dissociation constants K_d of the full-length HMGB1 and $\Delta 30$ variant proteins for the FAM-labeled four-way junction DNA J1. The buffer conditions were 10mM potassium phosphate (pH 7.4) and 150 mM NaCl. The dissociation constants K_d for the full-length HMGB1 and $\Delta 30$ variant proteins were 180 ± 10 nM and 15 ± 2 nM, respectively.

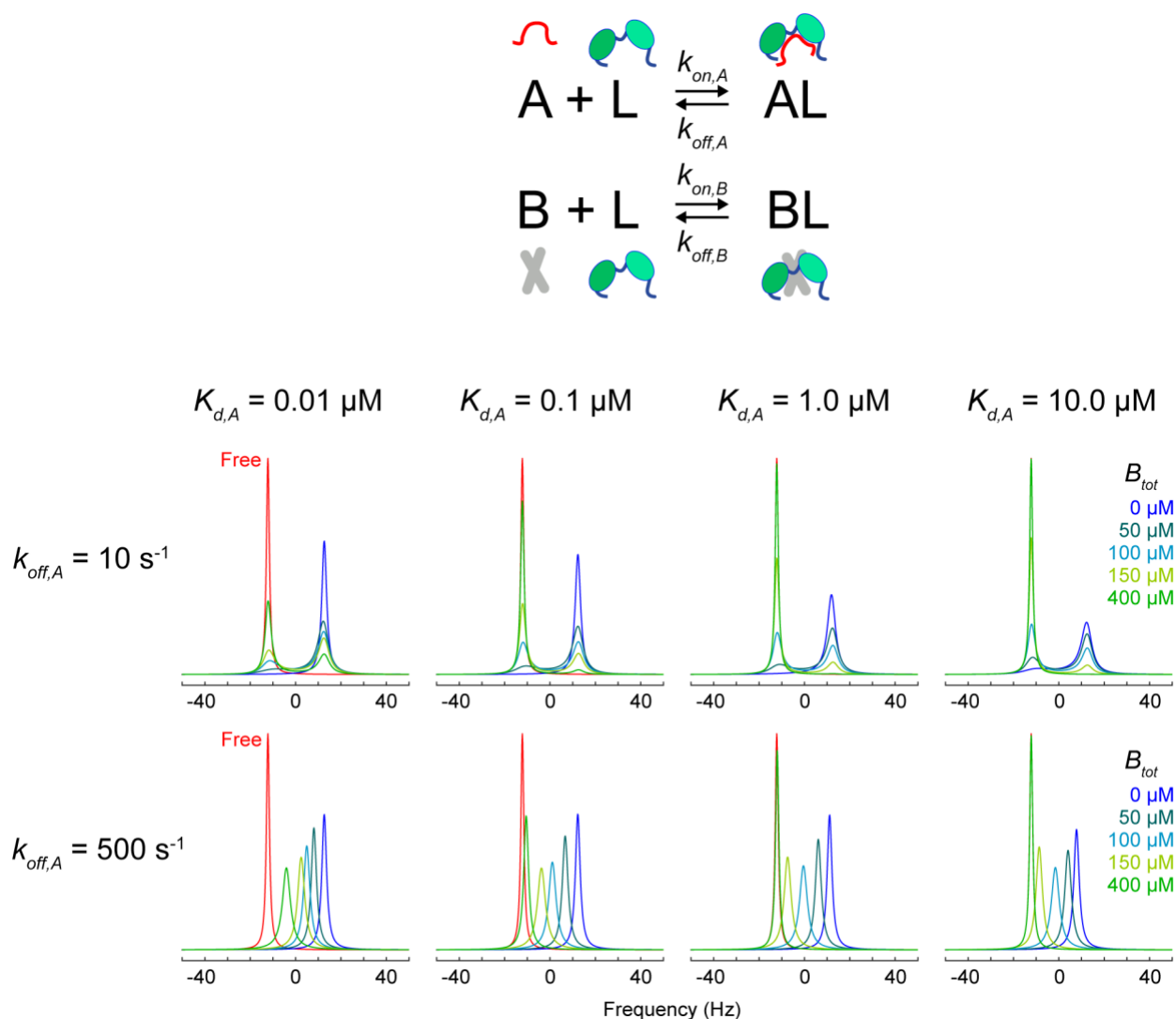


Figure S10. Bloch-McConnell equation-based simulations of the NMR-based competition data shown in Figure 4A. In the scheme shown above, the molecules A, B, and L correspond to ^{15}N -labeled DERT30, unlabeled DNA J1, and unlabeled $\Delta 30$ protein. ^{15}N NMR line shapes for ^{15}N DERT30 were simulated using Eqs. s9-s18 together with $A_{\text{tot}} = [\text{A}] + [\text{AL}] = 200 \mu\text{M}$, $L_{\text{tot}} = [\text{L}] + [\text{AL}] + [\text{BL}] = 200 \mu\text{M}$, $|\Omega_{\text{A}} - \Omega_{\text{AL}}|/(2\pi) = 25 \text{ Hz}$ (based on the data shown in Figure 4A), $R_{2,\text{A}} = 5 \text{ s}^{-1}$, $R_{2,\text{AL}} = 8 \text{ s}^{-1}$, $K_{d,\text{B}} = 0.015 \mu\text{M}$ (based on the results shown in Figure S9), and the indicated parameters. The simulations were performed using MATLAB scripts similar to those given in the Supporting Information of Sahu and Iwahara.⁴⁵ It should be noted that when the exchange between A and AL is in a fast exchange regime (e.g., with $k_{\text{off},\text{A}} = 500 \text{ s}^{-1}$), the apparent chemical shift of A (^{15}N DERT30) depends on the total concentration of the competitor molecule B (DNA J1) regardless of the timescale of the exchange between B and BL.

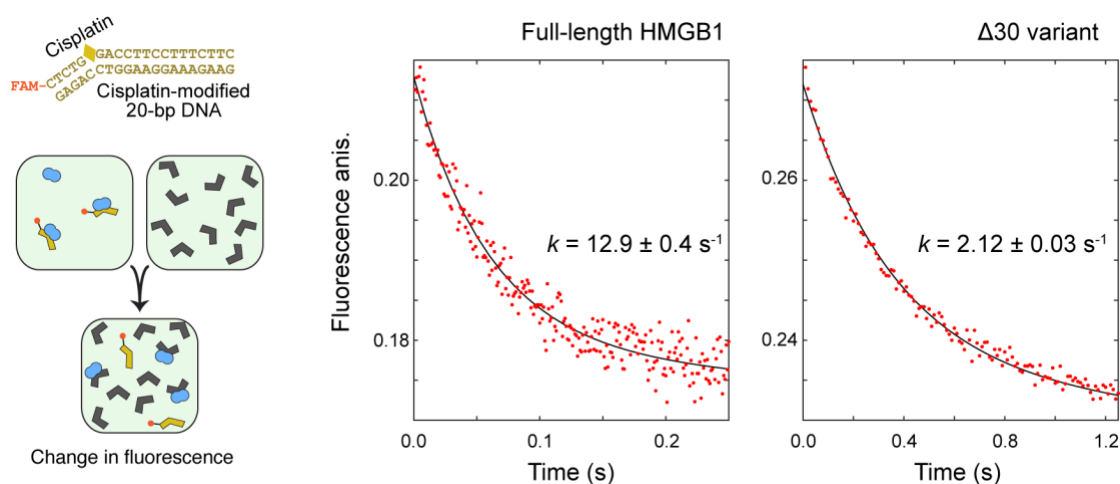


Figure S11. Stopped-flow fluorescence time-course data on the kinetics of protein dissociation from cisplatin-modified 20-bp DNA (cisGG20). To measure the rate constants reported in Figure 4B, a solution of a complex formed with 50 nM protein (either full-length HMGB1 or $\Delta 30$) and 10 nM FAM-labeled cisGG20 was mixed with a solution of unlabeled cisGG20. Shown here are fluorescence time-course data from the stopped-flow experiments with 1.75 μM unlabeled cisGG20. Because the dissociation kinetics of the full-length HMGB1 protein was remarkably faster, different time intervals were used for the two proteins. The time intervals were 1 ms for the full-length HMGB1 protein and 10 ms for the $\Delta 30$ variant. Note that the time range is different for the two graphs.

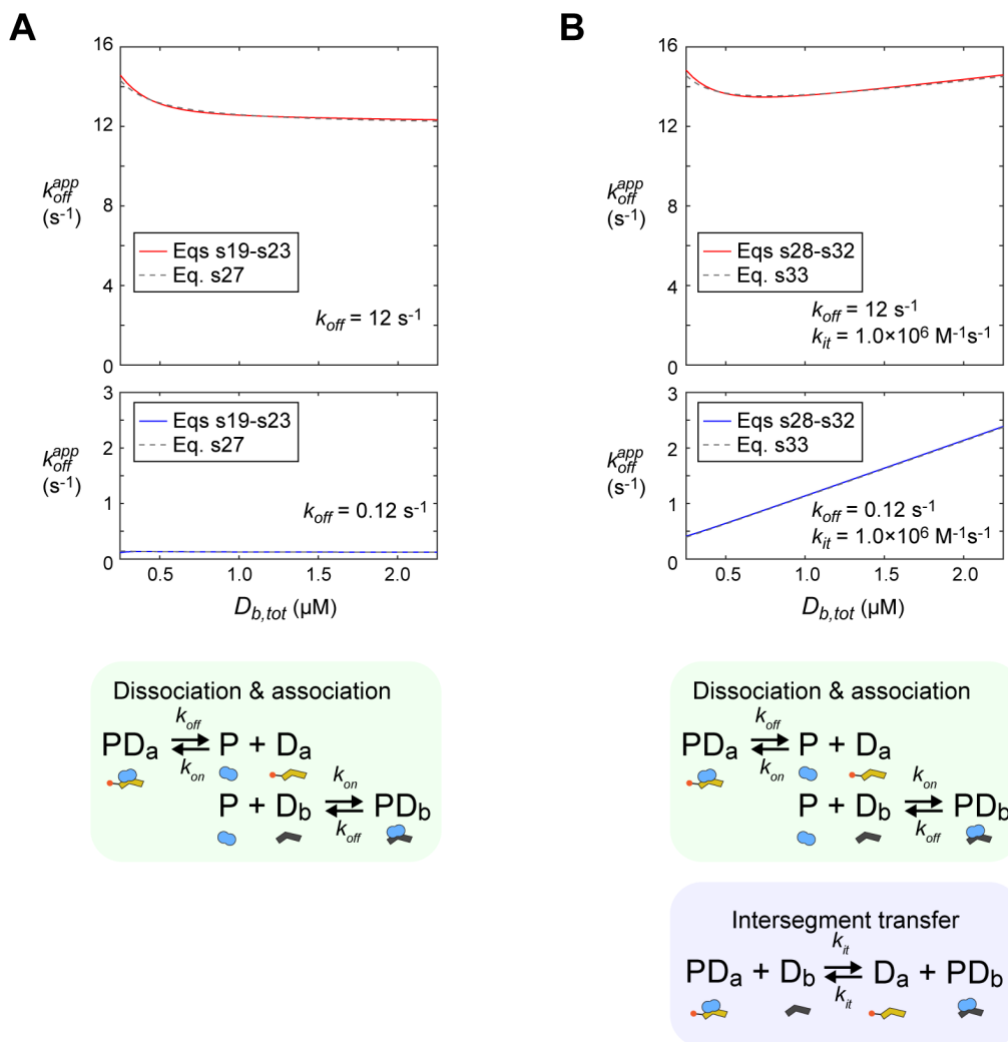


Figure S12. Simulations of the apparent dissociate rate constants in the stopped-flow experiment. Panel A shows the simulations for systems involving dissociation and association, whereas Panel B shows the simulations for systems involving intersegment transfer in addition to dissociation and association. The red and blue curves were obtained using the time course obtained with the rate equations (Eqs. s19-s23 for A and Eqs. s28-s32 for B). The rate equations were numerically solved using an ordinary-differential-equations solver ('ode23s') of the MATLAB software. The gray dotted lines were obtained with the approximate analytical expression (Eq. s27 for A and Eq. s33 for B). $P_{tot} = 50$ nM, $D_{a,tot} = 10$ nM, and $k_{on} = 1.2 \times 10^9$ M⁻¹ s⁻¹ were used along with the values of k_{off} , k_{it} , and $D_{b,tot}$ indicated in the graphs.

References for Supporting Information

1. Wang, X.; Greenblatt, H. M.; Bigman, L. S.; Yu, B.; Pletka, C. C.; Levy, Y.; Iwahara, J., Dynamic Autoinhibition of the Hmgb1 Protein Via Electrostatic Fuzzy Interactions of Intrinsically Disordered Regions. *J Mol Biol* **2021**, *433*, 167122.
2. Kallenbach, N. R.; Ma, R.-I.; Seeman, N. C., An Immobile Nucleic Acid Junction Constructed from Oligonucleotides. *Nature* **1983**, *305*, 829-831.
3. Wang, X.; Mayorga-Flores, M.; Bien, K. G.; Bailey, A. O.; Iwahara, J., DNA-Mediated Proteolysis by Neutrophil Elastase Enhances Binding Activities of the Hmgb1 Protein. *J Biol Chem* **2022**, *298*, 102577.
4. Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G., III; Rance, M.; Skelton, N. J., *Protein Nmr Spectroscopy: Principles and Practice*. 2 ed.; Elsevier Academic Press: Burlington, 2007.
5. Bermel, W.; Bertini, I.; Chill, J.; Felli, I. C.; Haba, N.; Kumar M. V, V.; Pierattelli, R., Exclusively Heteronuclear ^{13}C -Detected Amino-Acid-Selective Nmr Experiments for the Study of Intrinsically Disordered Proteins (Idps). *ChemBioChem* **2012**, *13*, 2425-2432.
6. Felli, I. C.; Pierattelli, R., ^{13}C Direct Detected Nmr for Challenging Systems. *Chem Rev* **2022**, *122*, 9468-9496.
7. Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A., Nmrpipe - a Multidimensional Spectral Processing System Based on Unix Pipes. *J Biomol NMR* **1995**, *6*, 277-293.
8. Lee, W.; Tonelli, M.; Markley, J. L., Nmrfam-Sparky: Enhanced Software for Biomolecular Nmr Spectroscopy. *Bioinformatics* **2015**, *31*, 1325-1327.
9. Yu, B.; Pletka, C. C.; Pettitt, B. M.; Iwahara, J., De Novo Determination of near-Surface Electrostatic Potentials by Nmr. *Proc Natl Acad Sci U S A* **2021**, *118*, e2104020118.
10. Yu, B.; Pletka, C. C.; Iwahara, J., Protein Electrostatics Investigated through Paramagnetic Nmr for Nonpolar Groups. *J Phys Chem B* **2022**, *126*, 2196-2202.
11. Yu, B.; Wang, X.; Iwahara, J., Measuring Local Electrostatic Potentials around Nucleic Acids by Paramagnetic Nmr Spectroscopy. *J Phys Chem Lett* **2022**, *13*, 10025-10029.
12. Schwieters, C. D.; Bermejo, G. A.; Clore, G. M., Xplor-Nih for Molecular Structure Determination from Nmr and Other Data Sources. *Protein Sci* **2018**, *27*, 26-40.
13. Lipsitz, R. S.; Sharma, Y.; Brooks, B. R.; Tjandra, N., Hydrogen Bonding in High-Resolution Protein Structures: A New Method to Assess Nmr Protein Geometry. *J Am Chem Soc* **2002**, *124*, 10621-10626.

14. Grishaev, A.; Bax, A., An Empirical Backbone-Backbone Hydrogen-Bonding Potential in Proteins and Its Applications to Nmr Structure Refinement and Validation. *J Am Chem Soc* **2004**, *126*, 7281-7292.
15. Bermejo, G. A.; Clore, G. M.; Schwieters, C. D., Smooth Statistical Torsion Angle Potential Derived from a Large Conformational Database Via Adaptive Kernel Density Estimation Improves the Quality of Nmr Protein Structures. *Protein Sci* **2012**, *21*, 1824-1836.
16. Kuszewski, J.; Gronenborn, A. M.; Clore, G. M., Improving the Packing and Accuracy of Nmr Structures with a Pseudopotential for the Radius of Gyration. *J Am Chem Soc* **1999**, *121*, 2337-2338.
17. Kohn, J. E.; Millett, I. S.; Jacob, J.; Zagrovic, B.; Dillon, T. M.; Cingel, N.; Dothager, R. S.; Seifert, S.; Thiyagarajan, P.; Sosnick, T. R., et al., Random-Coil Behavior and the Dimensions of Chemically Unfolded Proteins. *Proc Natl Acad Sci U S A* **2004**, *101*, 12491-12496.
18. Dolinsky, T. J.; Nielsen, J. E.; McCammon, J. A.; Baker, N. A., Pdb2pqr: An Automated Pipeline for the Setup of Poisson-Boltzmann Electrostatics Calculations. *Nucleic Acids Res* **2004**, *32*, W665-667.
19. Dolinsky, T. J.; Czodrowski, P.; Li, H.; Nielsen, J. E.; Jensen, J. H.; Klebe, G.; Baker, N. A., Pdb2pqr: Expanding and Upgrading Automated Preparation of Biomolecular Structures for Molecular Simulations. *Nucleic Acids Res* **2007**, *35*, W522-525.
20. Jurrus, E.; Engel, D.; Star, K.; Monson, K.; Brandi, J.; Felberg, L. E.; Brookes, D. H.; Wilson, L.; Chen, J.; Liles, K., et al., Improvements to the Apbs Biomolecular Solvation Software Suite. *Protein Sci* **2018**, *27*, 112-128.
21. Greenfeld, M.; Herschlag, D., Probing Nucleic Acid-Ion Interactions with Buffer Exchange-Atomic Emission Spectroscopy. In *Biophysical, Chemical, and Functional Probes of Rna Structure, Interactions and Folding: Part B*, 2009; pp 375-389.
22. Bai, Y.; Greenfeld, M.; Travers, K. J.; Chu, V. B.; Lipfert, J.; Doniach, S.; Herschlag, D., Quantitative and Comprehensive Decomposition of the Ion Atmosphere around Nucleic Acids. *J Am Chem Soc* **2007**, *129*, 14981-14988.
23. Das, R.; Travers, K. J.; Bai, Y.; Herschlag, D., Determining the Mg²⁺ Stoichiometry for Folding an Rna Metal Ion Core. *J Am Chem Soc* **2005**, *127*, 8272-8273.
24. Gebala, M.; Herschlag, D., Quantitative Studies of an Rna Duplex Electrostatics by Ion Counting. *Biophys J* **2019**, *117*, 1116-1124.
25. Gebala, M.; Bonilla, S.; Bisaria, N.; Herschlag, D., Does Cation Size Affect Occupancy and Electrostatic Screening of the Nucleic Acid Ion Atmosphere? *J Am Chem Soc* **2016**, *138*, 10925-10934.

26. Gebala, M.; Giambasu, G. M.; Lipfert, J.; Bisaria, N.; Bonilla, S.; Li, G.; York, D. M.; Herschlag, D., Cation-Anion Interactions within the Nucleic Acid Ion Atmosphere Revealed by Ion Counting. *J Am Chem Soc* **2015**, *137*, 14705-14715.
27. Pletka, C. C.; Nepravishta, R.; Iwahara, J., Detecting Counterion Dynamics in DNA-Protein Association. *Angew Chem Int Ed Engl* **2020**, *59*, 1465-1468.
28. Yu, B.; Bien, K. G.; Pletka, C. C.; Iwahara, J., Dynamics of Cations around DNA and Protein as Revealed by ^{23}Na Diffusion Nmr Spectroscopy. *Anal Chem* **2022**, *94*, 2444-2452.
29. Yu, B.; Bien, K. G.; Wang, T.; Iwahara, J., Diffusion Nmr-Based Comparison of Electrostatic Influences of DNA on Various Monovalent Cations. *Biophys J* **2022**, *121*.
30. Yu, B.; Pletka, C. C.; Iwahara, J., Quantifying and Visualizing Weak Interactions between Anions and Proteins. *Proc Natl Acad Sci U S A* **2021**, *118*, e2015879118.
31. Hayamizu, K.; Price, W. S., A New Type of Sample Tube for Reducing Convection Effects in Pgse-Nmr Measurements of Self-Diffusion Coefficients of Liquid Samples. *J Magn Reson* **2004**, *167*, 328-333.
32. Wu, D. H.; Chen, A. D.; Johnson, C. S., An Improved Diffusion-Ordered Spectroscopy Experiment Incorporating Bipolar-Gradient Pulses. *J Magn Reson, Ser A* **1995**, *115*, 260-264.
33. Holz, M.; Weingartner, H., Calibration in Accurate Spin-Echo Self-Diffusion Measurements Using ^1H and Less-Common Nuclei. *J Magn Reson* **1991**, *92*, 115-125.
34. Johnson, C. S., Diffusion Ordered Nuclear Magnetic Resonance Spectroscopy: Principles and Applications. *Prog NMR Spect* **1999**, *34*, 203-256.
35. Zandarashvili, L.; Nguyen, D.; Anderson, K. M.; White, M. A.; Gorenstein, D. G.; Iwahara, J., Entropic Enhancement of Protein-DNA Affinity by Oxygen-to-Sulfur Substitution in DNA Phosphate. *Biophys J* **2015**, *109*, 1026-1037.
36. Itoh, Y.; Murata, A.; Takahashi, S.; Kamagata, K., Intrinsically Disordered Domain of Tumor Suppressor P53 Facilitates Target Search by Ultrafast Transfer between Different DNA Strands. *Nucleic Acids Res* **2018**, *46*, 7261-7269.
37. Esadze, A.; Iwahara, J., Stopped-Flow Fluorescence Kinetic Study of Protein Sliding and Intersegment Transfer in the Target DNA Search Process. *J Mol Biol* **2014**, *426*, 230-244.
38. Doucleff, M.; Clore, G. M., Global Jumping and Domain-Specific Intersegment Transfer between DNA Cognate Sites of the Multidomain Transcription Factor Oct-1. *Proc Natl Acad Sci U S A* **2008**, *105*, 13871-13876.
39. Esadze, A.; Kemme, C. A.; Kolomeisky, A. B.; Iwahara, J., Positive and Negative Impacts of Nonspecific Sites During Target Location by a Sequence-Specific DNA-Binding Protein: Origin of the Optimal Search at Physiological Ionic Strength. *Nucleic Acids Res* **2014**, *42*, 7039-7046.

40. Kroenke, C. D.; Loria, J. P.; Lee, L. K.; Rance, M.; Palmer, A. G., Longitudinal and Transverse H-1-N-15 Dipolar N-15 Chemical Shift Anisotropy Relaxation Interference: Unambiguous Determination of Rotational Diffusion Tensors and Chemical Exchange Effects in Biological Macromolecules. *J Am Chem Soc* **1998**, *120*, 7905-7915.
41. Tjandra, N.; Szabo, A.; Bax, A., Protein Backbone Dynamics and N-15 Chemical Shift Anisotropy from Quantitative Measurement of Relaxation Interference Effects. *J Am Chem Soc* **1996**, *118*, 6986-6991.
42. Pelupessy, P.; Espallargas, G. M.; Bodenhausen, G., Symmetrical Reconversion: Measuring Cross-Correlation Rates with Enhanced Accuracy. *J Magn Reson* **2003**, *161*, 258-264.
43. Goddard, T. D.; Huang, C. C.; Meng, E. C.; Pettersen, E. F.; Couch, G. S.; Morris, J. H.; Ferrin, T. E., Ucsf ChimeraX: Meeting Modern Challenges in Visualization and Analysis. *Protein Sci* **2018**, *27*, 14-25.
44. Zandarashvili, L.; Sahu, D.; Lee, K.; Lee, Y. S.; Singh, P.; Rajarathnam, K.; Iwahara, J., Real-Time Kinetics of High-Mobility Group Box 1 (Hmgb1) Oxidation in Extracellular Fluids Studied by in Situ Protein Nmr Spectroscopy. *J Biol Chem* **2013**, *288*, 11621-11627.
45. Sahu, D.; Iwahara, J., Discrete-State Kinetics Model for Nmr-Based Analysis of Protein Translocation on DNA at Equilibrium. *J Phys Chem B* **2017**, *121*, 9548-9556.