Supplementary Information for
De novo determination of near-surface electrostatic potentials by NMR

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Supplementary Information Text

1. Additional information about Eq. 1

Here, we consider paramagnetic relaxation enhancement (PRE) for macromolecular $^1$H nuclei due to their dipole-dipole interactions with unpaired electrons of paramagnetic cosolutes in the same solution. For this type of PRE, which is often referred to as solvent PRE (1), the paramagnetic relaxation rates depend on the concentration of the paramagnetic cosolutes, the diffusional properties of the macromolecule and the cosolutes, and the electron relaxation time (2, 3). According to the Solomon-Bloembergen theory (4, 5), the PRE rate $\Gamma_2$ for the transverse $^1$H nuclear magnetization is given by:

$$\Gamma_2 = 4 \frac{G^2}{15} \frac{\mu_B^2}{4\pi a^2} g^2 P(S + 1) \left[ f(0) + \frac{3}{4} f(\omega_H) \right]$$

where $\mu_B$ is the vacuum permeability; $\gamma$ is the $^1$H nuclear gyromagnetic ratio; $g$ is the electron $g$-factor; $\mu_B$ is the Bohr magneton; $S$ is the electron spin quantum number for the paramagnetic group ($S = \frac{1}{2}$ for PROXYL); and $\omega_H$ is the $^1$H Larmor frequency in rad s$^{-1}$ units. Recently, Okuno, Szabo, and Clore advanced the theory for solvent PRE (2). According to their theory, the spectral density function $J(\omega)$ has a value at the zero frequency as follows:

$$J(0) = \frac{N_c}{(r^{-6})}$$

$N_c$ is the number of cosolute molecules; $\gamma$ is the correlation time for the dipole-dipole interaction between the macromolecular $^1$H nuclear spin and the cosolute unpaired electron spin; $r$ is the distance between the spins; and $<r^{-6}>$ represents the ensemble average of $r^6$. The correlation time $\gamma$ depends on the diffusion coefficient of the cosolute, the rotational correlation time of the macromolecule, and the electron relaxation time (2, 3). Explicit expression of $\gamma$ is model-dependent, but that is not important for our current method because $\gamma$ is canceled out in Eq. 3 in the main text. With the potential of mean force $U(r)$, in which $r$ represents the position vector for the unpaired electron spin with respect to the $^1$H spin, the ensemble average $<r^{-6}>$ is given by (2):

$$<r^{-6}> = V^{-1} \int_0^\infty 4\pi r^{-4} \exp \left( -\frac{U(r)}{kT} \right) dr$$

where $V$ represents the volume. $J(\omega_H)$ can be approximated by:

$$J(\omega_H) = \frac{J(0)}{(1+a\omega_H+b\sqrt{\omega_H})^2}$$

in which $a$ and $b$ are empirically determined parameters (2). Eq. 1 in the main text is obtained through combining Eqs. s1-4 together with $N_c V = 1000 Na c_p$, where $Na$ is Avogadro’s number and $c_p$ is the concentration of the paramagnetic cosolute in mol/L units. Thus, the parameter $\xi$ in Eq. 1 is:

$$\xi = 200Na \left( \frac{\mu_B^2}{4\pi a^2} \gamma g^2 \mu_B^2 \right) \left[ 1 + \frac{3}{4} \frac{1}{(1+a\omega_H+b\sqrt{\omega_H})^2} \right]$$

For PROXYL derivatives, $a$ and $b$ were reported to be $\sim 6 \times 10^{-10} s$ and $\sim 2 \times 10^{-6} s$, respectively (2). With $a$ and $b$ on these orders, the first term in the square brackets is predominant in Eq. s5. Therefore, the parameter $\xi$ for PRE $\Gamma_2$ data is insensitive to variations in the parameters $a$ and $b$. This nature of $\Gamma_2$ rates is important for de novo determination of near-surface electrostatic potentials because the ratio $\Gamma_2/S\Gamma_2$ is unaffected even if values of $a$ and $b$ are different for amino-methyl-PROXYL and carboxy-PROXYL.

We should point out that PRE rates $\Gamma_1$ for longitudinal $^1$H nuclear magnetizations are less suitable for our current method. The equations for the $\Gamma_1$ rate and its coefficient corresponding to $\xi$ are as follows:

$$\Gamma_1 = \frac{2 \pi}{5} \left( \frac{\mu_B^2}{4\pi a^2} \gamma g^2 \mu_B^2 \right) \left[ 1 + \frac{3}{4} \frac{1}{(1+a\omega_H+b\sqrt{\omega_H})^2} \right]$$

Unlike $\Gamma_2$ data, as Eq. s7 suggests, $\Gamma_1$ data are relatively sensitive to variations in the parameters $a$ and $b$, which may be slightly different between amino-methyl-PROXYL and carboxy-PROXYL. Due to this problem, $\Gamma_1$ data are less suited for the de novo determination of near-surface electrostatic potentials.
2. Diffusion measurements for PROXYL derivatives in the reduced form

The PRE correlation time $t_c$ in Eq. 1 in the main text depends on the diffusional properties of PROXYL derivatives. To investigate the diffusional properties of amino-methyl-PROXYL and carboxy-PROXYL molecules, we used NMR to measure the diffusion coefficients for the reduced forms of these compounds, which have a hydroxylamine group instead of a nitroxide radical (Fig. S1a). This reduction can readily be achieved by using ascorbic acids (6) and it converts each paramagnetic molecule into a diamagnetic molecule, allowing us to observe $^1$H NMR signals for the diffusion measurements. The reduced PROXYL derivatives were made by adding ascorbic acid to solutions of 20 mM amino-methyl or carboxy-PROXYL. The final concentrations were 40 mM for ascorbic acid and 16 mM for a PROXYL derivative. For each diffusion measurement, 11 gradient strengths were used: 1.0, 6.1, 11.1, 16.1, 21.1, 26.1, 31.2, 36.2, 41.2, 46.2, and 51.2 gauss/cm. The pulsed field gradients were calibrated using the self-diffusion of liquid N,N-dimethylformamide (DMF) at 25°C as a reference, for which the diffusion coefficient is $1.63 \times 10^{-5}$ cm$^2$ s$^{-1}$. The diffusion coefficient $D$ was determined through fitting calculations using the following relationship between the signal intensity $I$ and the pulse field gradient $G$ (8):

$$I = I_0 \exp[-Dy^2G^2\delta^2(\Delta - \delta/3 - \tau/2)]$$

where $\delta$ is the total length of a pair of bipolar gradients; $\Delta$, the time between the beginning points of two spin echo periods; and $\tau$, the time between two gradients in each spin echo. The determined values of the diffusion coefficient $D$ are shown in Fig. S1b. The diffusion coefficients $D$ were virtually identical for amino-methyl-PROXYL and carboxy-PROXYL. This supports our assumption for Eq. 3 (main text) that the correlation time $t_c$ is identical for solvent PRE rates $\Gamma_{2,+}$ and $\Gamma_{2,-}$.

3. Optimization of the accessibility radius for the paramagnetic center

When Eqs. 3 and 4 in the main text are used to calculate $\phi_{ENS}^{PB}$ potentials, the grid points inaccessible for the PROXYL paramagnetic center should be excluded. For this exclusion, we used the criterion of $d_{min} < r_{VDW} + r_{pc}$, where $d_{min}$ is the distance to the closest macromolecular atom; $r_{VDW}$ is its van der Waals radius; and $r_{pc}$ is the effective radius that defines the accessibility of the PROXYL paramagnetic center. To empirically optimize $r_{pc}$, we examined RMSDs between the experimental $\phi_{ENS}$ data for rigid regions of ubiquitin (PDB 1UBQ) and the corresponding $\phi_{ENS}^{PB}$ data obtained using various $r_{pc}$ values (Fig. S3). The smallest RMSD was found when $r_{pc} = 3.5$ Å. This value of $r_{pc}$ seems reasonable, considering that the nitroxide group of the PROXYL group is surrounded by four methyl groups. Based on these results, we used $r_{pc} = 3.5$ Å for all other calculations of $\phi_{ENS}^{PB}$.

4. $\phi_{ENS}^{PB}$ potentials for structure ensembles

We calculated $\phi_{ENS}^{PB}$ potentials for backbone H$_N$ atoms using the ensemble of 10 NMR structures of ubiquitin (PDB code 1D32)(9). Fig. S4a shows the averages and standard deviations of $\phi_{ENS}^{PB}$ potentials for individual residues. Larger variations were observed in the loop regions and the C-terminal tail. To further investigate impacts of structural fluctuation, we also generated 100 structures with altered side-chain conformers and loop backbone conformations while keeping the backbone atoms fixed at the coordinates of the 1.8 Å resolution crystal structure (1UBQ) (10). The structures were generated with the Xplor-NIH software (11) using a simulating annealing protocol in a torsion angle space under the influence of conformational database potentials (12). Fig. S4b shows the average and standard deviation of $\phi_{ENS}^{PB}$ potentials for individual residues in these 100 structures.
Fig. S1. Diffusion data on the reduced forms of amino-methyl-PROXYL and carboxy-PROXYL. To observe NMR signals without the influence of paramagnetic relaxation enhancement, the compounds were reduced with ascorbic acid. (a) Chemical structures of the reduced forms of the PROXYL derivatives. (b) NMR-based diffusion data for the reduced forms of amino-methyl-PROXYL and carboxy-PROXYL. Uncertainties in diffusion coefficients represent standard errors of means for three replicates. See Section 2 in Supplementary Text for additional information.

| Compound         | $D \text{ (cm}^2 \text{s}^{-1})$ |
|------------------|----------------------------------|
| Reduced amino-methyl-PROXYL | $(5.20 \pm 0.02) \times 10^{-6}$ |
| Reduced carboxy-PROXYL      | $(5.45 \pm 0.02) \times 10^{-6}$ |
Fig. S2. Example of $^1$H 1D NMR spectrum for the reduced form of amino-methyl-PROXYL for quantification of PROXYL compounds. Integrals for the $^1$H signals from two methyl groups of DMSO and four methyl groups of completely reduced amino-methyl-PROXYL (diamagnetic) are indicated. To observe intensities representing the Boltzmann equilibrium, the $^1$H spectrum was recorded with a single scan without any dummy scans. See Materials and Methods for experimental details.
Fig. S3. RMSD between the experimental $\phi_{ENS}$ data and computational $\phi_{ENS}^{PB}$ data as a function of the accessibility radius $r_{pc}$ for the PROXYL paramagnetic center. Experimental data for 53 NH groups located in rigid regions and the computational data for the 1.8-Å resolution crystal structure of ubiquitin (PDB 1UBQ) were used. Based on the order parameters $S^2$ determined by Tjandra et al. (13), the $\phi_{ENS}$ potential data for NH groups for which the order parameter $S^2 < 0.8$ were excluded in order to avoid a bias due to conformational flexibility or structural uncertainties. See Section 3 in Supplementary Text for additional information.
Fig. S4. The effective near-surface potentials \( \phi_{\text{ENS}} \) predicted for H\(_{N}\) atoms using structure ensembles. (a) Results for the 10 NMR structures of ubiquitin (PDB code 1D3Z). Each green bar indicates the average and the standard deviation of the \( \phi_{\text{ENS}} \) potentials calculated for the NMR structure ensemble. (b) Results for 100 structures generated from the 1.8-Å resolution crystal structure (PDB code 1UBQ) by varying side-chain conformers and loop backbone conformations through simulated annealing under the influence of conformational database potentials (12) while keeping the secondary structure backbone atoms fixed. Each green bar indicates the average and the standard deviation of the \( \phi_{\text{ENS}} \) potentials calculated for the ensemble of 100 structures. In each panel, experimental \( \phi_{\text{ENS}} \) data and the \( \phi_{\text{ENS}} \) potentials predicted for the crystal structure 1UBQ at the ionic strength of 30 mM are also shown in red and blue, respectively. See Section 4 in Supplementary Text for additional information.
Fig. S5. Effective near-surface electrostatic potentials $\phi_{ENS}$ determined from NMR PRE data for ubiquitin at pH 7.5 and the ionic strength of 130 mM. (a) $^1$H PRE rates $\Gamma_2$ measured with cationic and anionic PROXYL derivatives (10 mM). (b) Experimental $\phi_{ENS}$ potentials (red) determined from the PRE data for individual residues. Theoretical effective near-surface electrostatic potentials $\phi_{ENS}^P$ (blue) and $\phi_{ENS}^{PB}$ (green) predicted from the 1.8-Å resolution crystal structure (PDB 1UBQ) are also plotted (see the main text for the definitions). (c) Correlation between the experimental $\phi_{ENS}$ data and the theoretical $\phi_{ENS}^{PB}$. Data points for the secondary structure regions are shown in black and those for loop regions are shown in gray.
Fig. S6. Impact of variation in the protein interior dielectric constant $\varepsilon_i$ on the prediction of effective near-surface electrostatic potentials $\phi_{ENS}^{PB}$ for ubiquitin (PDB 1UBQ) at pH 7.5 and the ionic strength of 30 mM. (a) $\phi_{ENS}^{PB}$ potentials calculated for $^{1}H_N$ nuclei of ubiquitin with $\varepsilon_i = 2$, 4, 10, and 20. This set covers a typical range of $\varepsilon_i$ for protein interior regions (14). All other parameters were set as described in the main text. (b) Correlation between $\phi_{ENS}^{PB}$ potentials calculated with $\varepsilon_i = 2$ and those calculated with $\varepsilon_i = 20$. The RMSD was 1.1 mV. Linear regression is represented by a red line, with a slope of 0.91.
Table S1. PRE $\Gamma_2$ rates for backbone $^1{\text{H}}_N$ nuclei of ubiquitin at the ionic strength of 30 mM

| Residue | $\Gamma_2,\; (s^{-1})^a$ | $\Gamma_2,\; (s^{-1})^b$ | Residue | $\Gamma_2,\; (s^{-1})^a$ | $\Gamma_2,\; (s^{-1})^b$ |
|---------|-------------------------|-------------------------|---------|-------------------------|-------------------------|
| Q 2     | 1.37 ± 0.07             | 1.64 ± 0.07             | Q 41    | 1.31 ± 0.08             | 2.35 ± 0.08             |
| I 3     | 1.13 ± 0.10             | 1.06 ± 0.09             | R 42    | 1.55 ± 0.09             | 5.02 ± 0.08             |
| F 4     | 1.10 ± 0.11             | 1.88 ± 0.10             | L 43    | 1.23 ± 0.10             | 4.10 ± 0.09             |
| V 5     | 1.09 ± 0.10             | 1.78 ± 0.09             | I 44    | 2.34 ± 0.10             | 10.92 ± 0.10            |
| K 6     | 1.50 ± 0.09             | 4.96 ± 0.09             | F 45    | 2.25 ± 0.09             | 10.00 ± 0.09            |
| T 7     | 1.85 ± 0.07             | 4.46 ± 0.07             | A 46    | n.d. 3                  | 1.71 ± 0.07             |
| L 8     | 16.65 ± 1.53            | 26.69 ± 1.86            | G 47    | 14.88 ± 0.16            | 64.43 ± 0.64            |
| T 9     | n.d. 1                  | n.d. 1                  | K 48    | 3.31 ± 0.07             | 15.01 ± 0.07            |
| G 10    | n.d. 3                  | 8.01 ± 0.69             | Q 49    | 14.55 ± 0.14            | 51.48 ± 0.40            |
| K 11    | -0.75 ± 0.21            | 4.98 ± 0.22             | L 50    | 1.56 ± 0.10             | 5.45 ± 0.09             |
| T 12    | n.d.                    | 13.97 ± 1.46            | E 51    | 1.97 ± 0.10             | 3.66 ± 0.09             |
| I 13    | 0.96 ± 0.10             | 2.01 ± 0.09             | D 52    | 8.35 ± 0.07             | 33.38 ± 0.10            |
| T 14    | 3.94 ± 0.09             | 8.75 ± 0.08             | G 53    | n.d. 1                  | n.d. 1                  |
| L 15    | 0.79 ± 0.09             | 1.16 ± 0.08             | R 54    | 2.67 ± 0.07             | 1.79 ± 0.07             |
| E 16    | 9.01 ± 0.09             | 5.30 ± 0.08             | T 55    | 2.22 ± 0.09             | 1.76 ± 0.09             |
| V 17    | 0.86 ± 0.08             | 0.59 ± 0.07             | L 56    | 0.80 ± 0.09             | 0.88 ± 0.09             |
| E 18    | 1.86 ± 0.09             | 0.81 ± 0.08             | S 57    | 1.29 ± 0.06             | 1.12 ± 0.06             |
| S 20    | 2.40 ± 0.08             | 0.98 ± 0.07             | D 58    | 1.67 ± 0.08             | 1.50 ± 0.07             |
| D 21    | 1.71 ± 0.07             | 0.76 ± 0.07             | Y 59    | 1.62 ± 0.09             | 2.93 ± 0.09             |
| T 22    | 4.49 ± 0.09             | 1.15 ± 0.08             | N 60    | 1.57 ± 0.08             | 3.86 ± 0.08             |
| I 23    | 1.14 ± 0.10             | 0.83 ± 0.09             | I 61    | 1.34 ± 0.08             | 2.05 ± 0.08             |
| E 24    | n.d. 3                  | n.d. 3                  | Q 62    | 1.43 ± 0.08             | 2.42 ± 0.07             |
| N 25    | 3.90 ± 0.09             | 0.79 ± 0.08             | K 63    | 12.90 ± 0.10            | 20.69 ± 0.10            |
| V 26    | 2.10 ± 0.08             | 0.86 ± 0.08             | E 64    | 1.24 ± 0.11             | 1.71 ± 0.10             |
| K 27    | 1.71 ± 0.09             | 1.19 ± 0.08             | S 65    | 0.97 ± 0.07             | 1.19 ± 0.06             |
| A 28    | 4.61 ± 0.07             | 1.71 ± 0.07             | T 66    | 11.67 ± 0.10            | 20.88 ± 0.10            |
| K 29    | 5.47 ± 0.08             | 1.76 ± 0.08             | L 67    | 1.00 ± 0.11             | 3.15 ± 0.11             |
| I 30    | 2.49 ± 0.10             | 1.42 ± 0.09             | H 68    | 2.52 ± 0.10             | 12.26 ± 0.10            |
| Q 31    | 2.47 ± 0.08             | 1.74 ± 0.08             | L 69    | 2.15 ± 0.10             | 9.69 ± 0.09             |
| D 32    | 4.28 ± 0.07             | 1.65 ± 0.06             | V 70    | 2.13 ± 0.10             | 10.14 ± 0.09            |
| K 33    | 2.83 ± 0.07             | 1.48 ± 0.06             | L 71    | 11.25 ± 0.08            | 32.50 ± 0.11            |
| E 34    | 3.00 ± 0.10             | 1.81 ± 0.09             | R 72    | 1.51 ± 0.08             | 9.02 ± 0.08             |
| G 35    | 7.15 ± 0.10             | 2.62 ± 0.09             | L 73    | 5.46 ± 0.48             | 41.71 ± 1.12            |
| I 36    | 4.36 ± 0.08             | 4.12 ± 0.07             | R 74    | n.d. 1                  | n.d. 1                  |
| D 39    | 2.63 ± 0.08             | 0.75 ± 0.07             | G 75    | n.d. 1                  | n.d. 1                  |
| Q 40    | 1.77 ± 0.09             | 2.28 ± 0.08             | G 76    | 1.38 ± 0.05             | 5.31 ± 0.05             |

$^a$Solvent PRE arising from 10 mM amino-methyl-PROXYL; $^b$Solvent PRE arising from 10 mM carboxyl-PROXYL; $^1$The signal was too broad; $^2$PRE was too large to measure; $^3$Error was too large.
Table S2. PRE $\Gamma_2$ rates for backbone $^1$H nuclei of ubiquitin at the ionic strength of 130 mM

| Residue | $\Gamma_2^+ \text{ (s}^{-1}\text{)}^a$ | $\Gamma_2^- \text{ (s}^{-1}\text{)}^b$ | Residue | $\Gamma_2^+ \text{ (s}^{-1}\text{)}^a$ | $\Gamma_2^- \text{ (s}^{-1}\text{)}^b$ |
|---------|---------------------------------|---------------------------------|---------|---------------------------------|---------------------------------|
| Q 2     | -0.04 ± 0.10                   | 0.17 ± 0.11                     | Q 41    | 1.03 ± 0.12                    | 1.50 ± 0.12                     |
| I 3     | 0.78 ± 0.14                    | 0.77 ± 0.15                     | R 42    | 1.34 ± 0.13                    | 3.19 ± 0.13                     |
| F 4     | 0.98 ± 0.16                    | 1.32 ± 0.17                     | L 43    | 0.60 ± 0.15                    | 2.29 ± 0.15                     |
| V 5     | 0.82 ± 0.15                    | 1.28 ± 0.16                     | I 44    | 2.69 ± 0.15                    | 7.82 ± 0.16                     |
| K 6     | 1.44 ± 0.14                    | 3.26 ± 0.14                     | F 45    | 2.23 ± 0.14                    | 7.11 ± 0.15                     |
| T 7     | 1.13 ± 0.10                    | 2.86 ± 0.11                     | A 46    | 1.46 ± 4.77                    | 9.22 ± 6.19                     |
| L 8     | 13.22 ± 1.21                   | 20.83 ± 1.43                    | G 47    | 17.43 ± 0.21                   | 40.21 ± 0.48                    |
| T 9     | n.d.$^1$                       | n.d.$^1$                        | K 48    | 3.32 ± 0.10                    | 10.47 ± 0.11                    |
| G 10    | n.d.$^3$                       | 0.06 ± 0.58                     | Q 49    | 16.35 ± 0.18                   | 36.57 ± 0.37                    |
| K 11    | -0.43 ± 0.22                   | 0.78 ± 0.23                     | L 50    | 1.20 ± 0.14                    | 3.37 ± 0.14                     |
| T 12    | 3.09 ± 1.04                    | 6.45 ± 1.21                     | E 51    | 1.02 ± 0.14                    | 2.36 ± 0.14                     |
| I 13    | 0.72 ± 0.15                    | 0.95 ± 0.16                     | D 52    | 9.46 ± 0.10                    | 29.35 ± 0.16                    |
| T 14    | 2.31 ± 0.12                    | 5.83 ± 0.13                     | G 53    | n.d.$^1$                       | n.d.$^1$                        |
| L 15    | 0.41 ± 0.13                    | 0.39 ± 0.14                     | R 54    | 1.19 ± 0.10                    | 0.52 ± 0.11                     |
| E 16    | 6.97 ± 0.12                    | 5.05 ± 0.12                     | T 55    | 1.01 ± 0.13                    | 0.79 ± 0.14                     |
| V 17    | 0.32 ± 0.12                    | -0.06 ± 0.12                    | L 56    | 0.42 ± 0.13                    | 0.14 ± 0.14                     |
| E 18    | 1.07 ± 0.12                    | 0.86 ± 0.13                     | S 57    | 0.12 ± 0.09                    | 0.36 ± 0.10                     |
| S 20    | 0.42 ± 0.11                    | -0.49 ± 0.11                    | D 58    | 0.24 ± 0.11                    | 0.13 ± 0.11                     |
| D 21    | 0.69 ± 0.11                    | 0.18 ± 0.11                     | Y 59    | 0.89 ± 0.13                    | 1.43 ± 0.14                     |
| T 22    | 2.60 ± 0.12                    | 0.47 ± 0.13                     | N 60    | 0.62 ± 0.12                    | 2.82 ± 0.13                     |
| I 23    | 0.61 ± 0.14                    | 0.62 ± 0.15                     | I 61    | 0.61 ± 0.12                    | 1.13 ± 0.13                     |
| E 24    | n.d.$^3$                       | n.d.$^3$                        | Q 62    | 0.65 ± 0.11                    | 1.25 ± 0.11                     |
| N 25    | 2.03 ± 0.12                    | 0.27 ± 0.12                     | K 63    | 11.34 ± 0.13                   | 19.15 ± 0.17                    |
| V 26    | 1.25 ± 0.12                    | 0.90 ± 0.13                     | E 64    | 1.12 ± 0.16                    | 1.01 ± 0.16                     |
| K 27    | 1.23 ± 0.12                    | 0.81 ± 0.13                     | S 65    | -0.03 ± 0.10                   | 0.06 ± 0.10                     |
| A 28    | 3.68 ± 0.11                    | 1.54 ± 0.11                     | T 66    | 12.39 ± 0.14                   | 15.64 ± 0.16                    |
| K 29    | 4.48 ± 0.12                    | 1.81 ± 0.12                     | L 67    | 0.96 ± 0.17                    | 2.13 ± 0.17                     |
| I 30    | 1.58 ± 0.14                    | 0.70 ± 0.15                     | H 68    | 2.94 ± 0.15                    | 9.00 ± 0.16                     |
| Q 31    | 1.54 ± 0.12                    | 1.16 ± 0.13                     | L 69    | 2.28 ± 0.14                    | 7.26 ± 0.15                     |
| D 32    | 3.54 ± 0.10                    | 1.82 ± 0.11                     | V 70    | 2.69 ± 0.15                    | 7.39 ± 0.16                     |
| K 33    | 2.16 ± 0.10                    | 1.30 ± 0.10                     | L 71    | 12.80 ± 0.11                   | 25.04 ± 0.16                    |
| E 34    | 2.87 ± 0.14                    | 2.02 ± 0.14                     | R 72    | 0.79 ± 0.11                    | 4.93 ± 0.12                     |
| G 35    | 6.18 ± 0.15                    | 2.09 ± 0.15                     | L 73    | 7.97 ± 0.44                    | 28.94 ± 0.80                    |
| I 36    | 4.12 ± 0.11                    | 3.51 ± 0.12                     | R 74    | n.d.$^1$                       | n.d.$^1$                        |
| D 39    | 0.32 ± 0.10                    | n.d.$^3$                        | G 75    | n.d.$^1$                       | n.d.$^1$                        |
| Q 40    | 0.94 ± 0.13                    | 1.10 ± 0.13                     | G 76    | -0.60 ± 0.07                   | 0.50 ± 0.08                     |

$^a$Solvent PRE arising from 10 mM amino-methyl-PROXYL; $^b$Solvent PRE arising from 10 mM carboxyl-PROXYL;

1The signal was too broad; 2PRE was too large to measure; 3Error was too large.
Table S3. $\Gamma_2$ rates for Antp-DNA complex at the ionic strength of 30 mM

| Residue | $\Gamma_2^a (s^{-1})$ | $\Gamma_2^b (s^{-1})$ | Residue | $\Gamma_2^a (s^{-1})$ | $\Gamma_2^b (s^{-1})$ |
|---------|------------------------|------------------------|---------|------------------------|------------------------|
| K       | 23.24 ± 0.24           | 2.30 ± 0.16            | I       | 8.33 ± 0.17            | 3.61 ± 0.18            |
| R       | 19.62 ± 0.15           | 0.60 ± 0.11            | E       | 10.80 ± 0.15           | 5.12 ± 0.15            |
| G       | 35.62 ± 0.27           | 2.92 ± 0.13            | I       | 12.46 ± 0.19           | 2.59 ± 0.19            |
| R       | 22.09 ± 0.14           | 0.48 ± 0.10            | A       | 8.12 ± 0.14            | 3.32 ± 0.15            |
| Q       | 7.77 ± 0.19            | 0.20 ± 0.20            | H       | 21.42 ± 4.95           | 1.63 ± 4.87            |
| T       | 31.28 ± 0.20           | 3.05 ± 0.12            | A       | n.d.                   | n.d.                   |
| Y       | 5.25 ± 0.15            | -0.05 ± 0.16           | L       | 12.46 ± 0.20           | 3.99 ± 0.21            |
| T       | 12.23 ± 0.21           | 3.18 ± 0.21            | S       | 9.03 ± 0.14            | 4.80 ± 0.15            |
| R       | 38.40 ± 4.51           | 14.68 ± 2.68           | L       | 10.09 ± 0.15           | 4.77 ± 0.16            |
| Y       | 21.62 ± 0.20           | 11.33 ± 0.19           | T       | 18.14 ± 0.21           | 4.99 ± 0.20            |
| Q       | 26.67 ± 0.28           | 7.45 ± 0.23            | E       | n.d.                   | n.d.                   |
| T       | 8.47 ± 0.16            | 1.72 ± 0.16            | R       | 77.21 ± 0.99           | 18.85 ± 0.22           |
| L       | 13.72 ± 0.15           | 2.76 ± 0.15            | Q       | 36.16 ± 0.37           | 3.25 ± 0.23            |
| E       | 9.00 ± 0.17            | 2.81 ± 0.18            | I       | 8.06 ± 0.20            | 1.69 ± 0.21            |
| L       | 7.18 ± 0.22            | 2.01 ± 0.23            | K       | 11.27 ± 0.19           | 0.74 ± 0.19            |
| E       | 14.16 ± 0.19           | 2.99 ± 0.20            | I       | 13.15 ± 0.25           | -0.10 ± 0.24           |
| K       | 26.09 ± 0.19           | 6.32 ± 0.15            | W       | 3.36 ± 0.18            | -1.00 ± 0.20           |
| E       | 16.34 ± 0.19           | 3.02 ± 0.19            | F       | 3.06 ± 0.26            | -0.75 ± 0.28           |
| F       | 8.59 ± 0.23            | 1.53 ± 0.24            | Q       | 5.18 ± 0.20            | -1.00 ± 0.21           |
| H       | 18.83 ± 0.19           | 4.27 ± 0.18            | N       | 3.42 ± 0.19            | -0.97 ± 0.21           |
| F       | 16.53 ± 0.16           | 2.52 ± 0.15            | R       | 7.06 ± 0.18            | -0.93 ± 0.19           |
| N       | 12.84 ± 0.14           | 1.39 ± 0.14            | R       | 7.39 ± 0.22            | -0.39 ± 0.24           |
| R       | 12.71 ± 0.13           | 6.05 ± 0.11            | M       | 7.79 ± 0.19            | -0.41 ± 0.21           |
| Y       | 89.03 ± 1.16           | 11.43 ± 0.19           | K       | 12.08 ± 0.18           | 0.51 ± 0.18            |
| L       | 32.10 ± 0.37           | -0.40 ± 0.24           | W       | 13.33 ± 0.20           | 0.14 ± 0.20            |
| T       | 38.70 ± 0.35           | 0.58 ± 0.18            | K       | 21.62 ± 0.16           | 0.99 ± 0.13            |
| R       | n.d.                   | 5.19 ± 0.71            | K       | 20.96 ± 0.13           | 0.76 ± 0.11            |
| R       | 34.17 ± 0.24           | 8.19 ± 0.15            | E       | 22.26 ± 0.12           | 1.67 ± 0.10            |
| R       | 12.83 ± 0.17           | 1.82 ± 0.16            | N       | 20.38 ± 0.07           | 1.00 ± 0.06            |
| R       | 9.54 ± 0.16            | 1.17 ± 0.17            | n.d.    | n.d.                   | n.d.                   |

$^a$Solvent PRE arising from 10 mM amino-methyl-PROXYL; $^b$Solvent PRE arising from 10 mM carboxyl-PROXYL; $^1$The signal was too broad; $^2$PRE was too large to measure; $^3$Error was too large.
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