Supporting Information

The High Dielectric Constant of Staphylococcal Nuclease is Encoded in its Structural Architecture

Garrett B. Goh,[†] Bertrand García-Moreno E.,[§] Charles L. Brooks III*[,†,‡]

† Department of Chemistry, University of Michigan, 930 N. University, Ann Arbor, Michigan 48109, United States

§ Department of Biophysics, Johns Hopkins University, 3400 North Charles St, Baltimore, Maryland 21218, United States

‡ Biophysics Program, University of Michigan, 930 N. University, Ann Arbor, Michigan 48109, United States

** To whom correspondence should be addressed: brookscl@umich.edu*

METHODS

Model Systems. The reference protein used in this study is the highly stable Δ+PHS mutant of staphylococcal nuclease that includes five substitutions (P117G, H124L, S128, G50F and V51N) and deletion of residues 44-49. (Protein Data Bank, PDB accession code: 3BDC). Three mutants of the Δ+PHS reference protein that differ by a point mutation of Val-66 to Lys-66, Glu-66 and Asp-66 were investigated (PDB accession code: 3HZX, 1U9R, 2OXP). In our studies, we also used the wild type staphylococcal nuclease (PDB accession code: 1STN) as the control.

Simulation. The input structures used in the molecular dynamics (MD) simulations are crystal structures obtained from the aforementioned PDB files. For each residue-66 mutant of Δ+PHS, an alternate protonation state structure was created by manually patching the Lys-66 residue to its neutral deprotonated state to simulate the effects of an elevated pH environment. Similarly, the Glu-66 and Asp-66 residue were also patched to their neutral protonated state to simulate the effects of a lowered pH environment. MD simulations for both native and alternative protonation states were performed. MD simulations were performed within the CHARMM molecular dynamics program (version c35a),[1] using the CHARMM27 all-atom force field for proteins and GBSW implicit solvent model.[2,3] The SHAKE algorithm[4] was used in all simulations to restrain the hydrogen-heavy atom bond lengths and the time step used was 2 fs. For each system studied, an equilibration time of 100 ps was followed by a simulation of 9 ns.

Calculation of Protein Dielectric Constant. The dipole fluctuations of the systems under study were analyzed using the Kirkwood-Fröhlich theory of Dielectrics to determine the native dielectric constant of the protein. This approach is similar to that reported by Simonson and

Brooks.[5] Previous work has established that the choice of implicit or explicit solvent does not affect the outcome of the results.[6] The value of the calculated dielectric constant is related to the G-factor as derived from the Kirkwood-Fröhlich equation:

$$G = \frac{< \Delta M_p^2 >}{k_B T r_p^3} = \frac{(2\varepsilon_w + 1)(\varepsilon_p - 1)}{(2\varepsilon_w + \varepsilon_p)}$$

ΔM_p refers to the deviation of the protein dipole moment from its mean and the brackets represent an ensemble average, which is taken to be time average of the trajectory in our studies. T refers to the temperature that was maintained at a constant 298K, and r_p is the spherical radius of the protein, which is taken to be $(5/3)^{1/2}$ the time average of the radius of gyration obtained from the trajectory.[5] The dielectric constant of water and protein are ε_w and ε_p, respectively. The uncertainty of our calculated $<\Delta M_p^2>$, G factor and dielectric constant is computed as the standard deviation of the last 3 x 1ns batch averages. The spatially-resolved dielectric constant was calculated by a similar method as reported previously[5] using an analogous formula, where ε_1 is the value in the distance-dependent region and ε_2 is taken to be the calculated dielectric constant for the entire protein:

$$G = \frac{< \Delta M_p^2 >}{k_B T r_p^3} = \frac{(\varepsilon_1 - 1)[(1 + 2\varepsilon_2)(2\varepsilon_w + 2\varepsilon_2) - 2(r_1 / r_2)^3(\varepsilon_w + \varepsilon_2)(1 - \varepsilon_2)}{(\varepsilon_1 + 2\varepsilon_2)(2\varepsilon_w + 2\varepsilon_2) - 2(r_1 / r_2)^3(\varepsilon_w - \varepsilon_2)(\varepsilon_1 - \varepsilon_2)}$$

Analysis for Local Fluctuations and/or Structural Reorganization. Using a method adapted from Feng *et. al.*,[7] we calculated the average protein structure for the entire MD trajectory. To determine the presence of local fluctuations and/or structural reorganization, the mass-weighted fluctuations of each residue in terms of Cartesian coordinates and torsional (phi and psi) angles were obtained and comparison were made between the average MD structures of the various protein systems.

NMR Chemical Shift Calculations. Chemical shift values were calculated using SHIFTS.[8] The production run of the MD simulation was extended to 10 ns and repeated five times using different seed numbers. The average chemical shift value was calculated from these five simulation runs, and it was used for comparison with experimental data.

RESULTS & DISCUSSION

To determine the presence of local structural reorganization in our simulations, we calculated a RMSD residue plot between the ensemble average structure (as obtained from the

MD trajectory) to the respective crystals structure for the Δ+PHS and Δ+PHS/V66K mutant (**Figure S1a**). The results indicate that root-mean-square (RMS) position of the residues of Δ+PHS and Δ+PHS/V66K varies from their respective crystal structures in a similar manner. No residues exhibit markedly different deviations, which if they did would be indicative of some local reorganization in our MD simulation. To determine if there were any positional differences in the residues between various simulation runs, we also calculated a RMSD residue plot to compare the ensemble average structure of the Δ+PHS/V66K mutant to the ensemble average structure of Δ+PHS. By including the fluctuations of each residue in the figure, we observed that while there were some slight positional differences between Δ+PHS/V66K and Δ+PHS (**Figure S1b**), the positional fluctuations during the simulation are far greater in magnitude than the RMSD values. We conclude that our simulations did not produce any local reorganization phenomenon.

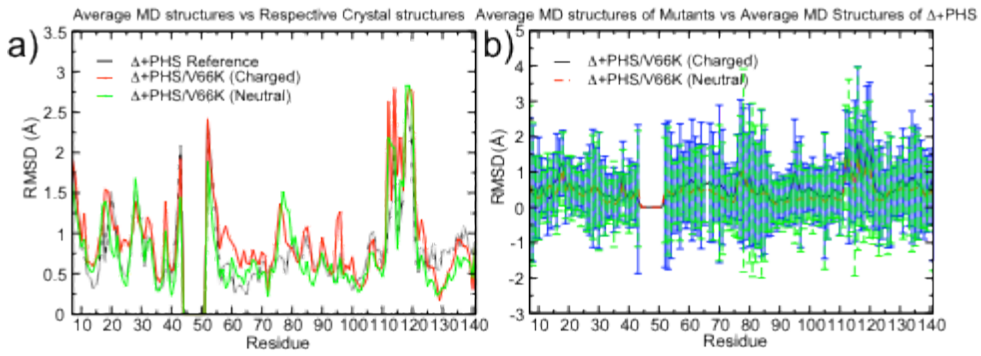

Figure S1: *RMSD residue plot that compares (a) the ensemble average structure as determined from the MD simulation to the respective crystal structure, (b) the ensemble average structure of the Δ+PHS/V66K mutant to the ensemble average structure of Δ+PHS (the error bars in the plot indicate the fluctuation of the residue).*

We looked for increased local fluctuations in our simulations by examining the root-mean-square fluctuations (in the Cartesian coordinates) per residue for Δ+PHS/V66K and Δ+PHS (**Figure S2**). There were no systematic patterns in which one state had consistently higher fluctuations than the others. There were minor variations in the fluctuations, most notably in the region of residues 15-20, 75-85 and 110-120. We suggest that the minor differences in fluctuations in residues 75-85 and 110-120 may be attributed to their location on the loop

adjoining secondary structure elements that are known to have increased flexibility due to the lack of neighboring constraints. For residues 15-20, we observed decreased fluctuations in the Δ+PHS/V66K mutant. Our simulations of Δ+PHS/V66K did not show any increased local fluctuations.

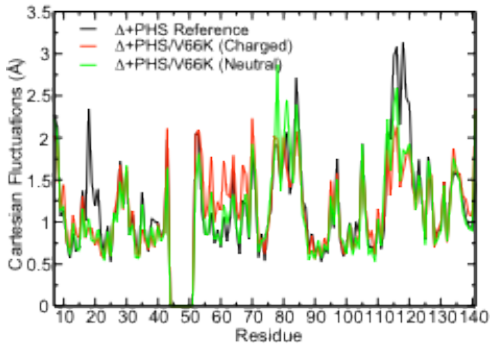

Figure S2: *Fluctuations per residue (in Cartesian coordinates) of Δ+PHS/V66K compared to Δ+PHS.*

While fluctuations (in Cartesian coordinates) would account for the change in chemical environment and thus support the NMR data reported by García-Moreno and co-workers, fluctuations in torsion angles (phi and psi) may also reflect a change in chemical environment. To investigate the possibility that the fluctuations may be explained in the form of twisting motions, we compared the average phi and psi angle values for Δ+PHS to Δ+PHS/V66K (**Figure S3**). No significant differences were observed. Plotting fluctuations (of phi and psi angles) per residue for Δ+PHS/V66K and Δ+PHS (**Figure S4**) yielded analogous findings to our earlier analysis of fluctuations in the Cartesian coordinates. Minor variations in the torsion angle fluctuations were observed in the same region of residues 15-20, 75-85 and 110-120. In addition, the region of residues 15-20 also had decreased phi and psi fluctuations.

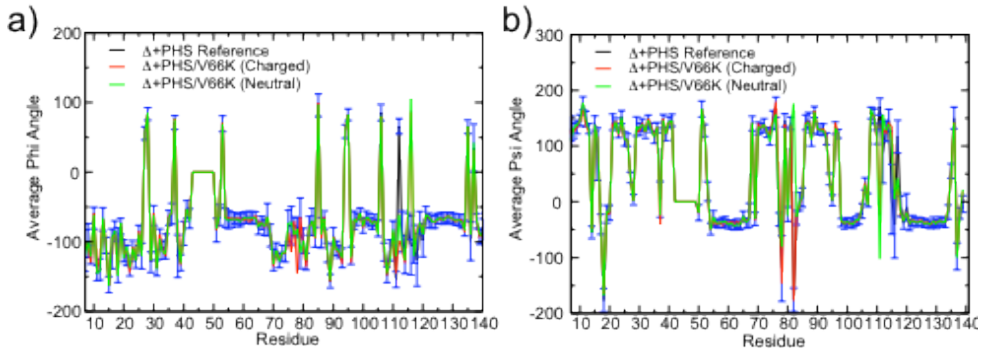

Figure S3: *Average (a) phi and (b) psi angles of Δ+PHS compared to Δ+PHS/V66K (the error bars in the plot indicate the torsion angle fluctuation of the residue).*

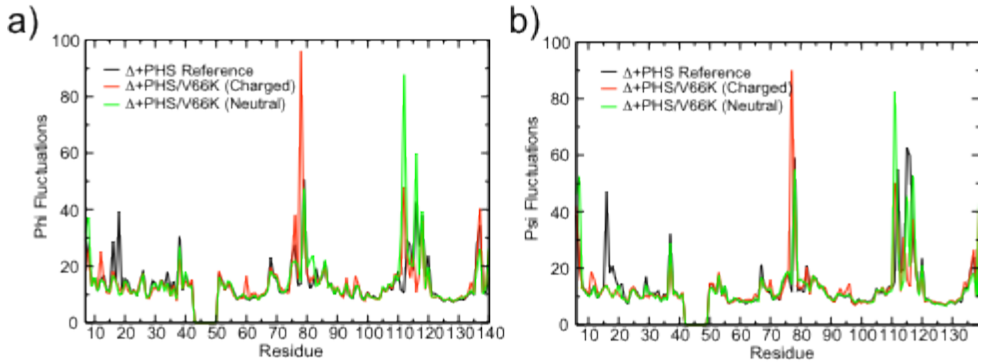

Figure S4: *Fluctuations per residue for (a) phi and (b) psi angles of Δ+PHS compared to Δ+PHS/V66K.*

COMPLETE REFERENCE

Complete Ref 17 in main manuscript listed as (1) below.

Garrett 10/18/11 9:20 AM
Comment:

Garrett 10/18/11 9:20 AM
Comment: Removed this section, since our simulations are extended to 9ns and there is no convergence issues with D+PHS

REFERENCES

(1) Brooks, B. R.; Brooks, C. L., III; Mackerell, A. D., Jr.; Nilsson, L.; Petrella, R. J.; Roux, B.; Won, Y.; Archontis, G.; Bartels, C.; Boresch, S.; Caflisch, A.; Caves, L.; Cui, Q.; Dinner, A. R.; Feig, M.; Fischer, S.; Gao, J.; Hodoscek, M.; Im, W.; Kuczera, K.; Lazaridis, T.; Ma, J.; Ovchinnikov, V.; Paci, E.; Pastor, R. W.; Post, C. B.; Pu, J. Z.; Schaefer, M.; Tidor, B.; Venable, R. M.; Woodcock, H. L.; Wu, X.; Yang, W.; York, D. M.; Karplus, M. *J Comput Chem* **2009**, *30*, 1545-614.

(2) Im, W.; Lee, M. S.; Brooks, C. L., III *J Comput Chem* **2003**, *24*, 1691-702.

(3) Chen, J.; Im, W.; Brooks, C. L., III *J Am Chem Soc* **2006**, *128*, 3728-36.

(4) van Gunsteren, W. F., Berendsen, H.J.C. *Mol. Phys.* **1977**, *34*, 1311-1327.

(5) Simonson, T.; Brooks, C. L., III *Journal of the American Chemical Society* **1996**, *118*, 8452-8458.
(6) Dominy, B. N.; Minoux, H.; Brooks, C. L., III *Proteins* **2004**, *57*, 128-41.
(7) Feng, J.; Wong, K. Y.; Lynch, G. C.; Gao, X.; Pettitt, B. M. *J Phys Chem B* **2009**, *113*, 9472-8.
(8) Moon, S.; Case, D. A. *J Biomol NMR* **2007**, *38*, 139-50.