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Supporting Material

Charge state of the globular histone core controls stability of the nucleosome.

Andrew T Fenley, David Adams, and Alexey V Onufriev

Supporting Material for "Charge state of the globular histone core controls stability of the nucleosome"

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Andrew T. Fenley[1],
David A. Adams[1,2],
Alexey Onufriev (alexey@cs.vt.edu)[1,3,4]

1. Department of Physics, Virginia Tech, Blacksburg, VA 24061
2. (Current Address) Department of Physics, University of Michigan, Ann Arbor, MI 48109
3. Department of Computer Science, Virginia Tech, Blacksburg, VA 24061

[4]Corresponding Author

1 Methodological details

1.1 Non-electrostatic contribution to ΔG: concentration dependence

The estimate for ΔG_{non} that we have made so far is, strictly speaking, only applicable at the experimental conditions of reference [1]. While a solution at pH=7.5 used in that experiment is a reasonable approximation for the environment inside the cell nucleus, the concentration of nucleosomes used in the experiment, $[C_{in\ vitro}] \sim 0.5\ \mu M$ may be quite different from what is relevant *in vivo*. To take this difference into account we define the nucleosome particle concentration dependent adjustment to ΔG_{non} as: $\Delta G_{non} \rightarrow \Delta G_{non} + \Delta\Delta G^{\dagger}$, where

$$\Delta\Delta G^{\dagger} = kTln\Big(\frac{[C_{in\ vivo}]}{[C_{in\ vitro}]}\Big) \tag{1}$$

We estimate the order of magnitude of $[C_{in\ vivo}]$ as follows. The total length of human DNA is $\sim 3m \sim 10^{10}$ base pairs (bp). Assuming the nucleus to be a sphere with a radius of $\sim 3\mu m$, assuming 200 bp per NCP, and assuming that most of eukaryotic DNA is wrapped on nucleosomes, we arrive at $C_{in\ vivo} \sim 300\mu M$. We note that this estimate is in fairly good agreement with the experimentally measured value in a HeLa cell of $140\mu M$ [2]. Substituting our estimate for $C_{in\ vivo}$ into equation 1 results in a relatively small correction of $\Delta\Delta G^{\dagger} \sim +3.7$ kcal/mol ($\sim 6kT$) to ΔG_{non} reported in the main text.

1.2 Parameter values for the idealized geometry model

We use $R_D = 10.9$ Å as the mean of the range (9.8 Å to 12.0 Å) suggested by Schellman and Stigter [3] for the effective electrostatic radius of the DNA. Others have used a similar value of $R_D = 10.0$ [4, 5]. The full length of the 147 bp DNA cylinder is $L_D = 490$ Å, corresponding to 3.32 Å/bp [6]. The NCP has a diameter of 105 Å and a length of $L_N = 57$ Å [7]. We estimate the radius of the histone octamer, $R_C = 30.7$ Å, as the radius of the NCP ($R_N = 52.5$ Å) minus the diameter of the DNA (21.8 Å). The solvent is modeled implicitly with a dielectric constant $\epsilon_{out} = 80$. The charge screening effects of monovalent salt are accounted for by the Debye-Hückel parameter, $\kappa = 0.329\sqrt{[salt]}$ [8, 5]. To account for the water trapped between the two wrapped helices being more ordered than free water, we use a dielectric constant of $\epsilon_{in} = 15$ for the wrapped DNA [9, 10].

We set the following parameters for estimating the charge state of the NCP: 0.8 M of monovalent salt, $\epsilon_{in} = 12.5$, $\epsilon_{out} = 80$, and a pH value of 7.5. The value of $\epsilon_{in} = 12.5$ was estimated as the volume averaged value between the DNA ($\epsilon_{in} = 15$) and the core ($\epsilon_{in} = 4$). The value of pH=7.5 was used in the experiments that observed the unfolding at 0.8 M of monovalent salt [1], and serves as a good estimate of the pH inside the nucleus [11]. The resulting total charge of the structure (Q_C + Q_D) is -199$|e|$. We then separated the DNA from the globular histone core (GHC) and determined the individual charge contributions. The DNA had a total charge: $Q_D = -292|e|$, and the GHC had a total charge of $Q_C = +93|e|$.

The fraction of the DNA's charge at the DNA-GHC interface is assumed to be equal to the fraction of DNA's surface area at the interface. To determine the fraction of DNA's

surface area at the interface, we use the cylindrical setup as shown in figure 2 of the main text. The surface area at the DNA-GHC interface is 11,640 Å^2, and the outer DNA surface area is 18,802 Å^2. Thus, the inner surface of the DNA accounts for 38% of the total surface area. We assume uniform charge distribution on the surface of the cylinder, excluding the ends, which results in $Q_{D1} = -292|e| \times 0.38 \approx -112|e|$. Finally, in all of the calculations we have accounted for ion exclusion effects with a standard Stern radius of $b = 2.0$Å. While many sources contribute to the error margin of ΔG within our model, the value is most sensitive to the uncertainty in the effective DNA radius, R_D. We estimate the corresponding error as half the difference between $\Delta G_{electro}$ computed with $R_D = 9.8$ and $R_D = 12.0$ Å [3].

1.3 Additional information for the atomistic model

To mimic the change in charge of a lysine residue that has been acetylated, we alter a subset of the lysine's atomic partial charges accordingly [12]. Table 1 shows in bold which partial charges of lysine were altered to change the total charge of the residue from +1 to 0.

Table 1: The conversion table for mimicking an acetylated lysine. The atoms with altered charges are shown in bold font.

Atom Type	Original Charge	Acetylated Charge
N	-0.348	-0.348
H	0.274	0.274
CA	-0.240	-0.240
HA	0.143	0.143
CB	-0.009	-0.009
2HB	0.036	0.036
3HB	0.036	0.036
CD	**-0.048**	**0.000**
2HD	**0.062**	**0.000**
3HD	**0.062**	**0.000**
CE	**-0.014**	**0.000**
2HE	**0.114**	**0.000**
3HE	**0.114**	**0.000**
CG	0.018	0.018
2HG	0.010	0.010
3HG	0.010	0.010
NZ	**-0.385**	**-0.075**
1HZ	**0.340**	**0.000**
2HZ	**0.340**	**0.000**
3HZ	**0.340**	**0.000**
C	0.734	0.734
O	-0.589	-0.589

To further test the claim that core residues have a substantially greater impact on $\Delta\Delta G$ than tail residues when altering localized charges, we performed four more computational

acetylation experiments as described in the main text, but this time choosing a different set of lysines to acetylate. Each residue was randomly chosen such that there would be one residue for each histone protein: H2AK75, H2BK31, H3K36, and H4K44. Overall, the $\Delta\Delta G$ values from these acetylated residues are similar to the values from the acetylated core resides from the main text, see table 2.

Table 2: The destabilization ($\Delta\Delta G$) of the nucleosome due to the acetylation (neutralization) of the lysines from the main text and a randomly selected lysine in the GHC from each histone protein (H2A, H2B, H3, and H4). The $\Delta\Delta G$ values are computed based on the full atomic level structure of the nucleosome using the numerical Poisson-Boltzmann equation (PBE) solver, as described in the main text. Also included are the non-linear PBE results (NLPBE) along with the linear PBE (LPBE) results. The analytical model would predict the ($\Delta\Delta G$) of acetylation for any pair of residues inside the core to be 30.8 kcal/mol.

Acetylated Lysines	LPBE: $\Delta\Delta G$ (kcal/mol)	NLPBE: $\Delta\Delta G$ (kcal/mol)
H3K56	8.7	5.0
H4K91	7.2	2.1
H2BK5	1.8	1.1
H3K4	0.07	0.03
H2AK75	10.6	6.5
H2BK31	15.3	8.8
H3K36	3.7	2.6
H4K44	13.3	9.5

2 Experimental bounds on absolute stability of the nucleosome

Observed partial detachment of DNA fragments off the GHC led to estimates of the contact energy per length of DNA to be $\approx 2.0\ kT$ per $1\ nm$ of the DNA length [13, 14]. Applying this to the full length of the wrapped DNA and adding a DNA bending cost of ~ 21 kcal/mol per wrapped turn of DNA [14] yields the total free energy favoring the *wrapped state* to be ~ 23 kcal/mol $\sim 40\ kT$. However, the fragments that "peeled off" in the experiment were limited to about 70 base pairs which is roughly half of the nucleosomal DNA. Since the strand-strand repulsion is largest in the compact conformation, the complete unwrapping of the nucleosomal DNA is expected to be relatively more unfavorable, per base-pair, than partial unwrapping. Thus, the above estimate could be considered as an approximate lower bound for true ΔG, consistent with our theoretical prediction.

An upper bound for the free energy of the *wrapped state* of NCP can be estimated from experiments involving pulling the DNA off the GHC by holding the nucleosome in place with an optical trap while the DNA attached to a cover slip is slowly moved away from the trap [15]. Here, the free energy of reversible dissociation of the first 76 bp was reported to be about 12 kcal/mol, while the cost of peeling off the remaining length of DNA was about 22 kcal/mol, yielding the total of 34 kcal/mol $\approx 60\ kT$. Since reversibility was not

achieved when the GHC dissociated from the DNA [15], this number can be considered an upper bound on the true ΔG. Our predicted value, see main text, is consistent with this estimate, within the error margin.

2.1 The physics of the nucleosome wrapping/ unwrapping: agreement with experiment

The model agrees with experiment on a number of observed trends and transitions in the nucleosome. We use our model to explain the physics behind the observed trends. Note, this section refers the reader to figure 3 in the Main Text when describing the different trends.

As expected, the nucleosome is in its "wrapped" state at physiological conditions indicated by the red dot in figure 3. Experimentally, its stability starts to gradually decrease [16, 1] as soon as the ionic strength (salt concentration) of the solution increases beyond the physiological value. When the salt concentration reaches about 0.8 M [NaCl] [1], the nucleosome is known to remain in the *unwrapped state*. These trends are clearly reproduced by the model: as the system moves away from the red dot towards higher salt concentrations, it approaches and eventually crosses the physical phase boundary into the *unwrapped state*. The physics behind this behavior is intuitively clear: an increase in the ionic strength of the solution screens out the favorable attraction between the positively charged GHC and the oppositely charged DNA. Within our model, the screening is controlled by the inverse Debye length $\kappa \propto \sqrt{[salt]}$; as it increases beyond the physiological value of $\kappa \approx 0.1\mathring{A}^{-1}$, the region of existence of the *wrapped state* of the nucleosome begins to shrink, see figure 3. Conversely, it was experimentally observed that a small decrease of the salt concentration from the physiological conditions leads to the increased stability ("freezing") of the structure [17]. Indeed, the predicted region of the *wrapped state* of the nucleosome particle slightly to the left of the physiological conditions corresponds to a larger (more stable) $|\Delta G|$ value, see figure 3.

The nucleosome also becomes destabilized as the ionic strength is lowered well below the physiological regime, see figure 3. Such a transition might seem counterintuitive since a reduction in the total number of screening ions increases the affinity between the positively charged core and negatively charged DNA. However, the over-all stability is a fine balance between these favorable interactions and the like charge repulsion within the DNA that disfavors conformations in which the DNA is bent. This low salt transition is experimentally known to occur near a monovalent salt concentration of approximately 0.001 M [18], in qualitative agreement with our results, see figure 3.

The "charge coordinate", see figure 3 can be conveniently accessed experimentally by modulating the pH of the environment. Lowering the pH of the solution (and thus increasing the GHC charge) leads to an increase in the stability of the nucleosome [18, 19]. Our model predicts this intuitive behavior; an increase in GHC charge just beyond the physiological value, red dot in figure 3, results in increased stability of the nucleosome. However, contrary to intuition, the model predicts the nucleosome to begin to destabilize as one continues to increase the core charge (*e.g.,* decrease pH) well beyond the physiological value. This destabilization effect has also been observed experimentally [16].

Although the GHC charge and ionic strength of the environments are independent parameters within the model, their variations affect the stability of the nucleosome through the same general mechanism of altering the electrostatic interactions. Thus, one expects that destabilization caused by changing one of the parameters can be offset by appropriate adjustment of the other. Indeed, in experiments the nucleosome is unwrapped at low salt and physiological pH; however, decreasing the pH, hence increasing the core charge, drives the system back to the *wrapped state*. This transition was shown to occur at a monovalent salt concentration of 0.1 mM near pH 5 [18]. Our model qualitatively predicts this transition in the lower left region of the phase diagram, figure 3.

2.2 The physics behind the transitions in the nucleosome: quantitative details

The origins of the the nucleosome unwrapping at high salt concentrations can be seen directly from equation (10) in the Main Text. For the sake of argument, in the following discussion we neglect the small terms arising from the presence of the Stern layer, *i.e.,* set $b \to 0$.

1) The high salt limit. When $\kappa \gg 1$, the ratio of the modified Bessel functions of the second kind goes to 1. Now the entire $\Delta G_{electro}$ is composed of two terms. The first term is negative and inversely proportional to κ, and the second term is a positive, constant contribution from the trapped field effect, see equation 2. At physiological salt, $\kappa \approx 0.1$, $\Delta G_{electro}$ is overall negative and overwhelms the unfavorable non-electrostatic part in the total ΔG and the system remains in the *wrapped state*. However, as κ increases, the favorable term inversely proportional to κ decreases and the balance shifts towards the *unwrapped state*.

$$\Delta G_{electro}|_{\kappa \gg 1} \approx -\frac{1}{\kappa \epsilon_{out}} \left[\frac{(Q_C)^2}{L_N R_C} + \frac{(Q_D)^2}{L_D R_D} - \frac{(Q_C + Q_D)^2}{L_N R_N} \right] + \frac{(Q_C + Q_{D1})^2}{\epsilon_{in} L_N} ln \left(\frac{R_N}{R_C} \right) \tag{2}$$

2) The low salt limit. The unwrapping of the system at low salt concentrations comes from an interplay between the favorable interactions of the core and DNA and the like charge repulsion within the DNA. These opposing interactions can be seen within our model, which in the low salt regimes gives:

$$\Delta G_{electro}|_{\kappa \ll 1} \approx \frac{Q_D^2 \left(1 + 2Q_C/Q_D\right)}{\epsilon_{out} L_N} ln \left(\frac{1}{\kappa R_D} \right) \tag{3}$$

Equation (3) shows that in the small κ regime the sign and magnitude of the electrostatic contribution to the stability of the nucleosome is controlled by the ratio of DNA's total charge to the charge of the GHC. If the ratio $|Q_C|/|Q_D|$ is less than $1/2$, then, for small enough κ, $\Delta G_{electro} > 0$. This can be interpreted as the DNA's strand to strand repulsion overwhelming the attraction between the DNA and the oppositely charged GHC and thus favoring the *unwrapped state*. The linear dependence on the natural log of the salt concentration in equation (3) has been seen before in experiment and theory in a similar context [20, 21, 22, 23].

At physiological ionic strength, $\Delta G_{electro}$ is the dominant contribution to the total stability. Within our model, if one alters the value of Q_C such that $|Q_C|/|Q_D| \sim 0.31$ or less, the system will favor the *unwrapped state*. The physics responsible for this behavior can be seen from the second term in equation (10) from the Main Text:

$$\Delta G_{electro}^{trapped\ field} = \frac{(Q_C + Q_{D1})^2}{\epsilon_{in} L_N} \ln\left(\frac{R_N}{R_C}\right) \tag{4}$$

This contribution always favors the *unwrapped state*, but it is relatively small when the GHC charge approximately equals the magnitude of the DNA charge at the interface, $|Q_C| \approx |Q_{D1}|$. As discussed above, this is the case under physiological conditions which keeps the nucleosome stable. However, considerable deviation between the core charge and the charge of the DNA at the interface makes $\Delta G_{electro}^{trapped\ field}$ the dominant contribution compared to other terms in the full expression of $\Delta G_{electro}$: note the low ϵ_{in} in the denominator and $ln\left(\frac{R_N}{R_C}\right)$ which is not close to 0. As $|Q_C + Q_{D1}|$ becomes large, the always destabilizing $\Delta G_{electro}^{trapped\ field}$ eventually drives the system to the *unwrapped state*. The physical meaning of $\Delta G_{electro}^{trapped\ field}$ is that it describes the destabilizing free energy of the electric field created by the unbalanced charge at the core/DNA interface. Given the topology of the folded nucleosome, figure 2 in the main text, most of this field is trapped in the low dielectric region of the DNA bulk. Electrostatic models that treat the DNA as a charged string of zero thickness [24, 25, 26, 8] do not include the destabilizing effects of an electric field in the low dielectric bulk of the DNA. Therefore, it appears that these models lack a mechanism to account for the experimentally observed destabilization of the structure caused by a large core charge. Models that do account for non-zero thickness of the DNA, but do not explicitly consider the core-DNA dielectric boundary [27, 5] also miss the "trapped field" effect and are therefore unlikely to predict the above trend as well.

2.3 Stability sensitivity to globular core charge is robust to model assumptions

The origin of the extreme sensitivity to small changes in the total charge of the GHC near and at *in vivo* conditions lies in the *wrapped state* energy contribution to ΔG, specifically in a term corresponding to a trapped electric field inside the wrapped DNA. This term is proportional to $(Q_{D_1} + Q_C)^2/\epsilon_{in}$, where Q_C is the total (positive) charge of the GHC, Q_{D_1} is the (negative) charge of the DNA in contact with the core, and ϵ_{in} represents the low dielectric constant for the DNA bulk. When the system is near and at *in vivo* conditions, the sum of Q_{D_1} and Q_C is relatively small –maintaining stability. However, as Q_C changes, $|Q_C + Q_{D_1}|$ becomes large and eventually drives the system to the *unwrapped state*.

The following quantitative analysis confirms that the contribution from the trapped electric field inside the low dielectric bulk of the DNA dominates all of the other two terms in the model for parameter values closely around *in vivo* conditions.

$$\left(\frac{\partial \Delta G_{tot}}{\partial Q_C}\right) = \frac{2(Q_D+Q_C)}{\epsilon_{out}L_N}\left(\ln\left[\frac{R_N+b}{R_N}\right]+\frac{1}{\kappa(R_N+b)}\frac{K_0[\kappa(R_N+b)]}{K_1[\kappa(R_N+b)]}\right) + \frac{2(Q_{D1}+Q_C)}{\epsilon_{in}L_N}\ln\left[\frac{R_N}{R_C}\right] - \frac{2Q_C}{\epsilon_{out}L_N}\left(\ln\left[\frac{R_C+b}{R_C}\right]+\frac{1}{\kappa(R_C+b)}\frac{K_0[\kappa(R_C+b)]}{K_1[\kappa(R_C+b)]}\right) \quad (5)$$

Equation (5) shows the partial derivative of ΔG_{tot} with respect to the GHC charge, Q_C. The second term is the contribution from the trapped electric field and dominates the other two terms for parameter values near and at *in vivo* conditions. In fact, it remains the dominant term for values of Q_C above +119$|e|$ and below +96$|e|$ in the model. Since the trapped electric field only exists in the *wrapped state*, the predicted sensitivity to changes in the GHC charge should be robust relative to any type of unwrapped state that does not allow for the trapped field to persist.

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