# Prevalence of Temperature-Dependent Heat Capacity Changes in Protein-DNA Interactions

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ABSTRACT A large, negative $\Delta Cp$ of DNA binding is a thermodynamic property of the majority of sequence-specific DNA-protein interactions, and a common, but not universal property of non-sequence-specific DNA binding. In a recent study of the binding of Taq polymerase to DNA, we showed that both the full-length polymerase and its "Klentaq" large fragment bind to primed-template DNA with significant negative heat capacities. Herein, we have extended this analysis by analyzing this data for temperature-variable heat capacity effects ($\Delta\Delta Cp$), and have similarly analyzed an additional 47 protein-DNA binding pairs from the scientific literature. Over half of the systems examined can be easily fit to a function that includes a $\Delta\Delta Cp$ parameter. Of these, 90% display negative $\Delta\Delta Cp$ values, with the result that the $\Delta Cp$ of DNA binding will become more negative with rising temperature. The results of this collective analysis have potentially significant consequences for current quantitative theories relating $\Delta Cp$ values to changes in accessible surface area, which rely on the assumption of temperature invariance of the $\Delta Cp$ of binding. Solution structural data for Klentaq polymerase demonstrate that the observed heat capacity effects are not the result of a coupled folding event.

## INTRODUCTION

Determination of $\Delta Cp$ for a protein-DNA interaction involves measuring either the temperature dependence of $\Delta H$ directly (i.e., the definition of $\Delta Cp$), or measuring the temperature dependence of $\Delta G$ (the curvature of which defines the $\Delta Cp$). The $\Delta Cp$ of a protein-DNA interaction is generally assumed to be invariant with temperature, particularly over restricted temperature ranges, and empirically the use of a temperature-invariant $\Delta Cp$ often provides a good fit to experimental data. There is no a priori requirement that $\Delta Cp$ be temperature-invariant for any molecular process (e.g., see (1,2)). The general assumption of temperature invariance of $\Delta Cp$ is based both on empirical evidence that such variance is indeed small for solvent restructuring (2), and on calculations showing that for determination of many protein folding thermodynamic parameters, this assumption introduces no significant errors (3,4).

A few researchers, however, have extended analyses of their DNA-binding data to include a parameter for temperature variation of $\Delta Cp$ (a $\Delta\Delta Cp$ parameter). For example, Lundbäck et al. fit a non-sequence-specific protein-DNA interaction with a temperature-dependent $\Delta Cp$ (5). Milev et al. describe a temperature-dependent heat capacity ($\Delta\Delta Cp$) and suggest it is caused by linked structural changes with temperature (6). Most recently, in a characteristically precise and thorough study, Kozlov and Lohman document a $\Delta\Delta Cp$ for the binding of *Escherichia coli* SSB to single-stranded DNA that is also anion-dependent (7).

Determining whether $\Delta Cp$ is temperature-dependent for an interaction can be elusive as it requires high precision data over a large temperature range, and involves quantifying small amounts of curvature in plots of $\Delta H$ versus temperature or subtle asymmetries in plots of $\Delta G$ versus temperature. In some of the very few studies of individual protein-DNA reactions where temperature dependence of $\Delta Cp$ has been documented, there have been suggestions that this behavior might be a general phenomenon (e.g., (7)). In this short report, we show that a simultaneous comparative analysis of a large number of protein-DNA systems reveals a high prevalence of $\Delta\Delta Cp$ values of similar magnitude, adding to the evidence that, indeed, temperature dependence of the heat capacity of protein-DNA interactions may be quite general.

## MATERIALS AND METHODS

Determination of $\Delta\Delta Cp$: $\Delta\Delta Cp$ in these analyses is defined as the linear temperature dependence of $\Delta Cp$,

$$\Delta Cp(T) = \Delta Cp_{\mathrm{r}} + \Delta\Delta Cp(T - T_{\mathrm{r}}),$$

and can be obtained from $\Delta H$ versus $T$ data using the equation

$$\Delta H(T) = \Delta H_{\mathrm{r}} + \Delta Cp_{\mathrm{r}}(T - T_{\mathrm{r}}) + \Delta\Delta Cp\left[\left(\frac{T^2 - T_{\mathrm{r}}^2}{2}\right) - T_{\mathrm{r}}(T - T_{\mathrm{r}})\right],$$

where $\Delta Cp(T)$ is the heat capacity change at any temperature $T$, the $\Delta H(T)$ values are the binding enthalpies measured at different temperatures, and $\Delta Cp_{\mathrm{r}}$ and $\Delta H_{\mathrm{r}}$ are the fitted heat capacity change and enthalpy values at any chosen reference temperature $T_{\mathrm{r}}$. $\Delta H$ data for Taq/Klentaq are reproduced from Datta and LiCata (8). The enthalpy of binding of 63/70-mer primed-template DNA to Taq and Klentaq was determined as a function of temperature in a MicroCal VP-ITC in 10 mM Tris, 75 mM KCl, 5 mM $MgCl_2$, pH 7.9. Additional experimental details can be found in Datta and LiCata (8).

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For Gibbs-Helmholtz ($\Delta G$ versus $T$ data), $\Delta\Delta Cp$ is defined as above, and can be obtained from the equation

$$\Delta G(T) = \Delta H_r + \int_{T_r}^{T} \Delta Cp(T)dT - T\left[(\Delta S_r) + \int_{T_r}^{T} \frac{\Delta Cp(T)}{T} dT\right]$$
$$= \Delta H_r + \Delta Cp_r(T - T_r) + \Delta\Delta Cp\left[\left(\frac{T^2 - T_r^2}{2}\right) - T_r(T - T_r)\right] - T\frac{\Delta H_r}{T_r}$$
$$- \Delta Cp_r T\ln\left(\frac{T}{T_r}\right) - T\Delta\Delta Cp\left[(T - T_r) - T_r\ln\left(\frac{T}{T_r}\right)\right],$$

where $\Delta G(T)$ is the free energy change at each temperature $T$, and $\Delta Cp_r$, $\Delta H_r$, and $T_r$ are the fitted heat capacity change, enthalpy, and $T_r$ values ($T_r$ is at either temperature where $\Delta G = 0$). $\Delta G$ versus $T$ data for Taq/Klentaq are from Datta and LiCata (8) and are determined from fluorescence anisotropy-monitored binding of Taq and Klentaq to a 63/70-mer primed-template DNA in 10 mM Tris, 75 mM KCl, 5 mM $MgCl_2$, pH 7.9 buffer at the indicated temperatures. Additional experimental details are in Datta and LiCata (8). All nonlinear fits were performed using KaleidaGraph (Synergy Software) and/or Origin 5.0 (Microcal Software).

Small angle x-ray scattering (SAXS) measurements of *Rg* were performed at the Stanford Synchrotron Radiation Research Laboratory on beamline 1–4 in 10 mM Tris, 75 mM KCl, 5 mM $MgCl_2$, pH 7.9 at the indicated temperatures. The data were analyzed using Guinier plots where *Rg* values were determined from the linear portions of the plots (9,10), and/or using the full $P(r)$ distance distribution function (11). Both approaches yield equivalent results. *Rh* measurements were conducted using a Zetasizer Nano DLS (dynamic light scattering) instrument in 10 mM $KPO_4$, 250 mM KCl, 5 mM $MgCl_2$, pH 7.5 at the indicated temperature. The data were analyzed using the manufacturer's software. Protein concentration in both sets of measurements was ~5 mg/ml. The 25°C SAXS-determined *Rg* values have been published previously (12).

## RESULTS AND DISCUSSION

### ΔΔ*Cp* values for Taq and Klentaq polymerases

In a recent study of the thermodynamics of binding of Taq polymerase to DNA, we showed that both the full-length polymerase and its ''Klentaq'' large fragment domain bind to primed-template DNA with a heat capacity of −0.7 to −0.8 kcal/mole (8). A large, negative $\Delta Cp$ of DNA binding is a property of the majority of sequence-specific DNA-protein interactions (13). The results for Taq and Klentaq are among those indicating that a smaller magnitude, but still relatively large $\Delta Cp$ is a common, but not universal property of non-sequence-specific DNA binding (8,14). Herein, we extended this analysis of Taq and Klentaq by analyzing this data for temperature-variable heat capacity effects, or $\Delta\Delta Cp$. We find that both data sets return equivalent values of $\Delta\Delta Cp$.

The top panel in Fig. 1 shows $\Delta H$ versus $T$ data for full-length Taq and Klentaq polymerases, fit with and without inclusion of a $\Delta\Delta Cp$ term. The middle panel shows a similar analysis for $\Delta G$ versus $T$ data. By visual inspection, the fits appear nearly equivalent, but in both cases, including a $\Delta\Delta Cp$ term improves the $\chi^2$ of the fit (see Table 1). $\Delta\Delta Cp$ values determined for Taq and Klentaq range from −8 to −19 cal/mole $K^2$. In general, however, the error envelopes for the $\Delta\Delta Cp$ parameters for Taq or Klentaq are too large to establish them as statistically significant (see Table 1). What is intriguing, however, is:

1. The similarity of $\Delta\Delta Cp$ values obtained from the calorimetric determinations of $\Delta H$ versus temperature and the equilibrium-binding determinations of $\Delta G$ versus temperature, because these are very different types of experiments, involving different potential for systematic or experimental errors.
2. The inability to obtain a better fit to the data with a zero $\Delta\Delta Cp$.
3. The fact that these seemingly minute $\Delta\Delta Cp$ values result in relatively large excursions of $\Delta Cp$ when propagated over a few decades of temperature.

The bottom panel of Fig. 1 shows the resultant $\Delta Cp$ values for binding of Taq and Klentaq to DNA over the temperature range of 10–60°C. It is also notable that if we fit 4–5 of the highest temperature data points from Fig. 1, middle, to obtain a temperature-invariant $\Delta Cp$, that paralleling Fig. 1, bottom, we obtain a $\Delta Cp$ value that is ~0.5 kcal/mole K more negative than if we fit 4–5 of the lowest temperature data points. Despite all this circumstantial evidence, however, the presence of a $\Delta\Delta Cp$ for Taq and Klentaq remains statistically unverified.

### ΔΔ*Cp* in other protein-DNA interactions

To investigate this issue further, however, we similarly analyzed 47 additional protein-DNA interaction data sets from the scientific literature, from 21 different publications (5,6,15–33). Data sets where the protein clearly and identifiably begins unfolding at higher binding temperatures were not included (e.g., (34–37)). Data sets were included if the data extended across ~20°C or more, and if the quantitative data were available in tabulated form. If data sets already included identification of significant linked processes with their own $\Delta Cp$ values (e.g., 15), data were only used if ''corrected'' data were provided having had the effects of known linked processes subtracted. Most of the data sets used were $\Delta H$ versus temperature data (only a few were $\Delta G$ versus temperature). For most of these original data sets in isolation, especially where there are measurements at perhaps only a small number of temperatures, there would have been little justification for

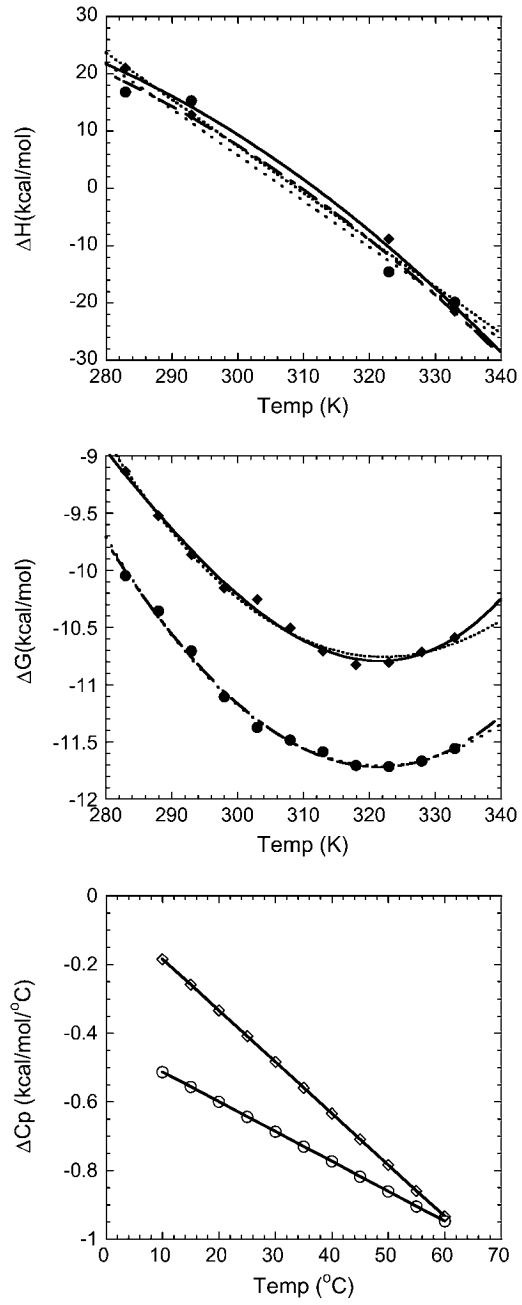


FIGURE 1 Fitting Taq and Klentaq DNA polymerase binding data with and without a $\Delta\Delta Cp$ parameter. (*Top panel*) The calorimetric $\Delta H$ for DNA binding by Taq (●) analyzed with (— —) and without (· · · ·) a $\Delta\Delta Cp$ parameter; and Klentaq (▲) analyzed with (—) and without (·····) a $\Delta\Delta Cp$ parameter. Data are from Datta and LiCata (8). (*Middle panel*) Gibbs-Helmholtz plot for DNA binding by Taq (●) analyzed with (— —) and without (· · · ·) a $\Delta\Delta Cp$ parameter; and Klentaq (▲) analyzed with (—) and without (·····) a $\Delta\Delta Cp$ parameter. Data are from Datta and LiCata (8). (*Bottom panel*) Calculated temperature dependence of the $\Delta Cp$ for DNA binding by Taq (●) and Klentaq (▲). The $\Delta\Delta Cp$ values used for this calculation are the means from the $\Delta H$ (*top panel*) and $\Delta G$ (*middle panel*) data sets (see Table 1).

testing for inclusion of a $\Delta\Delta Cp$ parameter. However, when examined in aggregate, some interesting patterns emerge.

Fig. 2 graphically depicts the fitted $\Delta\Delta Cp$ values found for 29 of the 49 data sets analyzed. Twenty-five data sets returned $\Delta\Delta Cp$ values in the approximate range of $\pm 30$ cal/mol $K^2$ (data sets *A–Y*), while four data sets returned somewhat larger $\Delta\Delta Cp$ values (data sets *a–d*). Table 1 summarizes the fit parameters for each of these 29 data sets. Fifteen of the 49 data sets were not fit better with addition of a $\Delta\Delta Cp$ parameter (these 15 data sets are not shown in Fig. 2 or in Table 1, but are listed in the legend to Fig. 2). In several cases, the same published study yielded some data sets that were fit better with a $\Delta\Delta Cp$ parameter and some data sets that were not (5,15,17–19,23).

Notable aspects of this analysis include: 1) the high prevalence of obtaining a better fit with addition of a $\Delta\Delta Cp$ parameter (29 of 49, or 59% of data sets); 2) the fact that most (26 of 29, or 90%) of the returned $\Delta\Delta Cp$ values are negative; and 3) the fact that the bulk of the $\Delta\Delta Cp$ values are of similar magnitude. If addition of the extra parameter were simply fitting experimental noise, one would expect approximately equal/random distribution of positive and negative $\Delta\Delta Cp$ values. If positive and negative $\Delta\Delta Cp$ values were equally likely, a simple binomial probability distribution calculation would predict the probability ($P(x)$) of finding the distribution in Fig. 2 as being $<0.0007\%$. I.e., if positive and negative $\Delta\Delta Cp$ values were equally probable ($p = 0.5$), then $P(x) = \binom{n}{x} p^{x}(1-p)^{(n-x)}$, where $n$ = number of trials and $x$ = number of negative $\Delta\Delta Cp$ values.

The fitted errors on $\Delta\Delta Cp$ for 7 of the 29 data sets shown in Fig. 2 indicate that the fitted $\Delta\Delta Cp$ values for those systems are statistically indistinguishable from zero (including, as mentioned above, our own data for Taq). The other data sets, however, return statistically significant $\Delta\Delta Cp$ values (two others barely make the cut). The $\Delta\Delta Cp$ values with large error envelopes are included here, however, because: 1) a comparably good fit for those data cannot be obtained by fixing the $\Delta\Delta Cp$ value at zero; and 2) the best fit $\Delta\Delta Cp$ value for those data sets match the pattern for the others. A distinguishing feature of meta-analysis, even in this simplified form, is the suggestion of patterns and correlations in large groups of data that are often not discernable and sometimes not statistically significant within the individual data sets. Even if these statistically borderline data sets are eliminated, the general conclusions of this analysis remain the same: a high percentage of the data sets analyzed are fit better with a negative $\Delta\Delta Cp$ parameter of similar magnitude and sign. Either this striking pattern is communicating information about $\Delta Cp$ behavior in protein-DNA interactions, or it is a highly improbable and coherent distribution of noise across a wide number of different experiments.

In Fig. 3, the mean $\Delta\Delta Cp$ value from data sets *A–Y* is used to illustrate the resultant change in $\Delta Cp$ versus

**TABLE 1 Data sets with fitted $\Delta\Delta Cp$ parameters**

| Protein-DNA interaction | Data, Fig. 2 | $\Delta\Delta Cp$ cal/mol K$^2$ | Temp range °C | $\chi 2$ with $\Delta\Delta Cp$ | $\chi 2$ without $\Delta\Delta Cp$ | $F^\dagger$ | Data ref. |
|---|---|---|---|---|---|---|---|
| *Pw*TBP-hairpin loop | A | −35.0 ± 12.9 | 15–45 | 6.559 | 22.768 | 7.41 | 16 |
| *Pw*TBP wt-20-mer | B | −16.4 ± 10.4 | 35–55 | 8.222 | 18.508 | 2.50 | 17 |
| *Pw*TBP E128A-20-mer | C | −15.8 ± 3.8 | 30–55 | 4.525 | 29.934 | 16.8 | 17 |
| *Pw*TBP E12AE128A-20-mer | D | −10.0 ± 9.9 | 25–45 | 7.473 | 11.279 | 1.02 | 17 |
| *Pw*TBP Q103E-20-mer | E | −22.5 ± 9.1 | 35–55 | 6.353 | 25.799 | 6.12 | 17 |
| *Pw*TBP Q103A-20-mer | F | −4.7 ± 10.5 | 30–50 | 8.437 | 13.212 | 4.73 | 17 |
| c-Myb R2R3*-MBS-I | G | −5.4 ± 5.3 | 12–30 | 0.089 | 0.135 | 1.03 | 18 |
| Sso 7d-poly(dGdC) | H | −4.5 ± 1.5[‡] | 15–45 | 0.025 | 0.227 | 8.27 | 5 |
| Sso 7d-poly(dGdC) | I | −4.1 ± <0.01[‡] | 16–35 | <0.001 | 0.022 | nd | 5 |
| Sox-5-10 bp | J | −20.9 ± 9.3 | 8–30 | 9.664 | 34.119 | 5.06 | 15 |
| vnd/NK-2 HD(wt)-18 bp | K | 12.0 ± 1.8 | 10–30 | 0.002 | 0.092 | 45.0 | 19 |
| GCN4-br-AP-1 | L | −2.9 ± 12.7 | 10–20 | 0.324 | 0.341 | 0.05 | 20 |
| GCN4-br-ATF/CREB | M | −3.6 ± 5.5 | 10–20 | 0.060 | 0.086 | 0.43 | 20 |
| *Mun*I-SP | N | 23.3 ± 15.9 | 14–30 | 3.956 | 15.474 | 2.91 | 21 |
| *Mun*I-SP | O | 6.6 ± 0.5 | 9–30 | 0.012 | 2.450 | 209.1 | 21 |
| *Mun*I-SP | P | −8.1 ± 4.3 | 13–30 | 0.298 | 2.954 | 8.91 | 21 |
| Oct-1 POU-DNA | Q | −9.4 ± 8.4 | 12–35 | 4.916 | 5.606 | 1.26 | 22 |
| Trp *repressor*-18 bp | R | −19.8 ± 15.7 | 10–40 | 11.701 | 16.357 | 1.59 | 23 |
| PU.1 ETS-λB | S | −11.5 ± <0.01 | 0–37 | 0.001 | 0.004 | nd | 24 |
| INT-DBD-13 bp | T | −18.9 ± 6.2 | 4–30 | 123.8 | 190.8 | 9.20 | 6 |
| Zfl-3-15 bp | U | −1.7 ± 3.9 | 13–45 | 3.482 | 4.181 | 0.20 | 25 |
| MetJ-12 bp | V | −17.0 ± <0.01 | 11–36 | 0.290 | 2.407 | nd | 26 |
| GR DBD-pGRE | W | −13.1 ± 3.6 | 10–34 | 0.298 | 1.314 | 13.6 | 27 |
| Taq-63/70-mer DNA ($\Delta H$) | X | −9.4 ± 23.7 | 10–60 | 22.609 | 26.200 | 0.16 | 8 |
| Taq-63/70-mer DNA ($\Delta G$) | X | −6.9 ± 9.0 | 10–60 | 0.016 | 0.018 | 0.58 | 8 |
| Klentaq-63/70-mer DNA ($\Delta H$) | Y | −11.1 ± 11.5 | 10–60 | 5.331 | 10.260 | 0.92 | 8 |
| Klentaq-63/70-mer DNA ($\Delta G$) | Y | −19.1 ± 10.0 | 10–60 | 0.020 | 0.030 | 3.67 | 8 |
| PU.1 ETS-λB | a | −68.5 ± 25.7 | 0–60 | 0.011 | 0.092 | 7.36 | 24 |
| PU.1 ETS-λB | b | −81.7 ± 28.1 | 0–50 | 0.021 | 0.163 | 13.5 | 24 |
| PurR-30 bp | c | −108 ± 238 | 1–37 | 0.006 | 0.981 | 162.5 | 28 |
| PurR-30 bp | d | −186 ± 34 | 1–37 | 0.138 | 0.167 | 0.42 | 28 |

[†]In the F-test, $F = ((\chi_1^2 - \chi_2^2)/\chi_2^2)/((dF_1 - dF_2)/dF_2)$, where $\chi_1^2$ and $\chi_2^2$ are the chi-squared values for the two different fits, and $dF_1$ and $dF_2$ are the degrees of freedom for each fit. *F*-values <1 indicate that the fit has not been improved by adding the new parameter beyond the statistical improvement expected from the reduction in degrees of freedom. *nd*, for some data sets *F* could not be reliably determined due to too few data points.
[‡]Lundbäck et al. previously reported a $\Delta\Delta Cp$ of −5 cal/K mol for these data (5).

temperature using an arbitrarily chosen starting $\Delta Cp$ of −0.5 kcal/mol K at 25°C. The standard deviation on the mean $\Delta\Delta Cp$ value from data sets *A–Y* was used to generate the dashed lines in the figure. The average net excursion of >−0.6 kcal/mole K over a 50°C range is a very large change of $\Delta Cp$—especially given that almost all $\Delta Cp$ values measured for protein-DNA interactions fall within a 0 to −2.0 kcal/mole K range.

## Temperature-induced compaction of Klentaq polymerase

One of the most popular current molecular explanations for a negative heat capacity change in a biomolecular process is the burial of nonpolar surface area (31,38–42). Although Klentaq polymerase does not thermally unfold until >100°C (43), one can still imagine a scenario where elevated temperature might induce an effective expansion or increase in dynamic fluctuation of the native state. In such a scenario, a hypothetically expanded native state might then need to recompact upon binding, thus increasing the net surface area burial upon DNA binding as the temperature increases. This hypothesis is similar to the coupled binding-plus-folding hypothesis (38), but adds a temperature-dependent effect. Fig. 4 empirically assays for such a possibility by directly measuring the effective size of native Klentaq polymerase as a function of increasing temperature. Instead of an expansion, however, both small angle x-ray scattering (SAXS) and dynamic light scattering (DLS) show that Klentaq polymerase compacts in size upon heating. While SAXS and DLS are both scattering techniques, they are, in fact, different methodologies, relying on completely different types of experimental signals and analyses. SAXS measures the static scattered intensity versus the angle of scattering, while DLS measures time-based, diffusion-induced fluctuations in scattering intensity. The two techniques are subject to different potential sources of systematic error, thus it is significant that they return similar measurements of the temperature-induced compaction of Klentaq. A similar temperature-induced native state compaction effect has also recently been documented for plasminogen (44). This result is interesting in its own right, and investigations of the potential origins of this

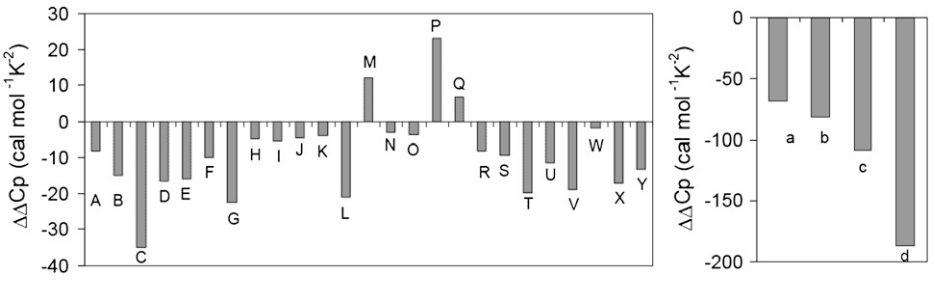


FIGURE 2 $\Delta\Delta Cp$ values for other protein-DNA interactions. $\Delta\Delta Cp$ values were obtained from the equations described in the text. The left panel includes data sets that fit better with a $\Delta\Delta Cp$ parameter in the range of $\pm 30$ cal/mol $K^2$ while the right panel includes data sets with larger $\Delta\Delta Cp$ values. These data sets are: (*A*) *Pw*TBP-hairpin loop (16); (*B–F*) *Pw*TBP wt-20-mer, *Pw*TBP E128A-20-mer, *Pw*TBP E12AE128A-20-mer, *Pw*TBP Q103E-20-mer, *Pw*TBP Q103A-20-mer, respectively (17); (*G*), c-Myb R2R3*-MBS-I (18); (*H* and *I*) Sso 7d-poly(dGdC) (5); (*J*) Sox-5-10 bp (15); (*K*) vnd/NK-2 HD(wt)-18 bp (19); (*L* and *M*) GCN4-br-AP-1, GCN4-br-ATF/CREB, respectively (20); (*N–P*) *Mun*I-SP (21); (*Q*) Oct-1 POU-DNA (22); (*R*) Trp *repressor*-18 bp (23); (*S*) PU.1 ETS-λB (24); (*T*) INT-DBD-13 bp (6); (*U*) Zfl-3-15 bp (25); (*V*) MetJ-12 bp (26); (*W*) GR DBD-pGRE (27); (*X*) Taq-63/70-mer DNA (average of $\Delta H$ and $\Delta G$ data from Table 1) (8); (*Y*) Klentaq-63/70-mer DNA (average of $\Delta H$ and $\Delta G$ data from Table 1) (8); (*a* and *b*) PU.1 ETS-λB (24); (*c* and *d*) PurR-30 bp (28). Data sets which were not fit better with a $\Delta\Delta Cp$ parameter are: one data set from Lundbäck et al. (5), two data sets from Privalov et al. (15), four data sets from Bergqvist et al. (17), one data set from Oda et al. (18), one data set from Gonzales et al. (19), one data set from Ladbury et al. (23), two data sets from Sieber and Allemann (29), one data set from Poon and Macgregor (30), and two data sets from Ha et al. (31) (total of 15 data sets that are not fit better with a $\Delta\Delta Cp$ parameter). Presence or absence of a fitted $\Delta\Delta Cp$ value could not be ascertained reliably for five of the data sets examined due to problematic fit diagnostics (such as indeterminate error envelopes for some parameters), these are: four data sets from Künne et al. (32) and one from Frank et al. (33).

unusual phenomenon will be the subject of future studies. For the purposes of the present investigation, however, Fig. 4 serves to demonstrate that coupled folding and binding surface area changes cannot account for a temperature-dependent change in $\Delta Cp$ for Klentaq (see also Potential Origins of $\Delta\Delta Cp$, below).

## Implications of a $\Delta\Delta Cp$

As mentioned in the Introduction, we are not the first investigators to find a temperature-dependent $\Delta Cp$ in their protein-DNA binding data. It is also likely that some of the investigators who do not mention such an effect in their studies could have fit for it and decided that the effect was too subtle to mention. What we have done, however, is analyze a large number of protein-DNA binding systems simultaneously and found that: 1), the majority of them (29 of 49) are fit better by including a temperature-dependent heat capacity; and 2), that the observed $\Delta\Delta Cp$ values are clearly nonrandom, with the majority of them (26 of 29) being negative in the binding direction. This means that the $\Delta Cp$ of binding for these 26 systems will become increasingly more negative as the temperature increases.

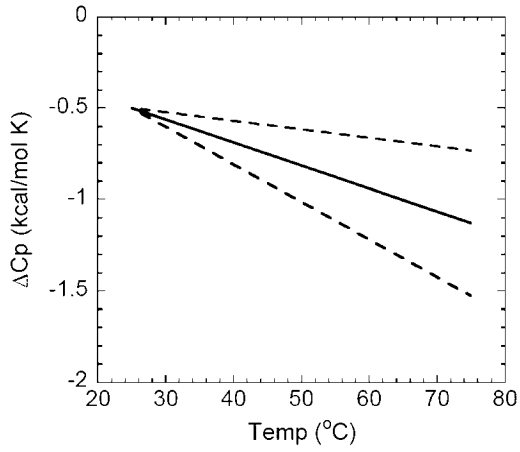


FIGURE 3 Illustration of the average change in $\Delta Cp$ for DNA binding that will occur as the temperature changes, given the $\Delta\Delta Cp$ values from Fig. 2. An idealized reference $\Delta Cp$ of $-0.5$ kcal/mol K at 25°C was chosen as a starting point. The $\Delta Cp$ represented by the solid line is calculated utilizing the mean $\Delta\Delta Cp$ values of data sets *A–Y* in Table 1 (mean $\Delta\Delta Cp = -0.013 \pm 0.008$ kcal/mol $K^2$). The dotted lines are $\Delta Cp$ values calculated using the $\pm$ standard deviation range on $\Delta\Delta Cp$ from data sets *A–Y* (i.e., *lower line* calculated with $\Delta\Delta Cp = -0.021$ kcal/mol $K^2$, *upper line* calculated with $\Delta\Delta Cp = -0.005$ kcal/mol $K^2$).

### $\Delta Cp$, $\Delta\Delta Cp$, and $\Delta ASA$

The question of whether $\Delta Cp$ is temperature-dependent is of interest because in many systems $\Delta Cp$ has been proposed to be correlated with structural information: most commonly the change in accessible surface area upon binding ($\Delta ASA$) (3,31,38–42). At least five different quantitative relationships

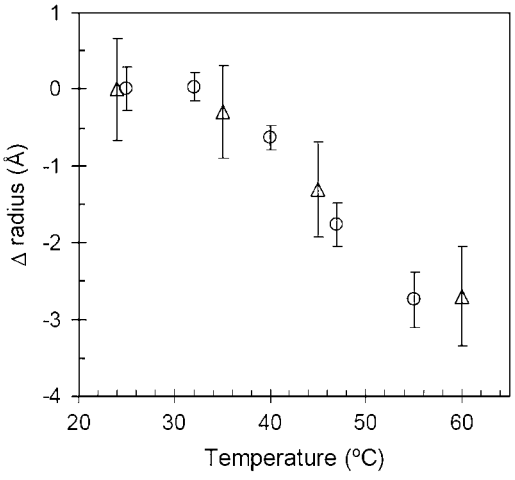


FIGURE 4 The change in the radius of gyration ($\Delta Rg$, *triangles*) and the hydrodynamic radius ($\Delta Rh$, *circles*) for Klentaq polymerase as a function of temperature.

between $\Delta Cp$ and the sum of buried nonpolar + polar surface areas have been proposed (3,39–42). All such relationships have the form: $\Delta Cp = -(x * \Delta ASA_{\text{non-polar}} - y * \Delta ASA_{\text{polar}})$, where $\Delta ASA_{\text{non-polar}}$ and $\Delta ASA_{\text{polar}}$ are the amounts of nonpolar and polar surface area buried in the interface, $x$ and $y$ are empirically determined constants, and $\Delta Cp$ is assumed to be temperature invariant. While these quantitative relationships continue to work reasonably well for protein folding, the increasing number of protein-DNA systems that deviate from these relationships (e.g., (13,23,45–47)) have led to proposals such as simultaneous folding plus binding (38) to account for such deviations. Coupled folding plus binding can be a difficult hypothesis to experimentally test. Some authors have definitively ruled out such an explanation for high $\Delta Cp$ values in some DNA-binding systems (5,6,23), while in other systems there is direct or crystal structure-based evidence for such an effect (22,33). Coupled binding and folding, however, does not account for either the value of $\Delta Cp$ at 25°C for Taq/Klentaq (8), or for the $\Delta\Delta Cp$ of binding. If burial of nonpolar surface area were the primary contributor to the negative $\Delta Cp$ of Taq-DNA binding, the average fitted $\Delta\Delta Cp$ value would correspond to >5000 Å$^2$ of additional surface area burial that would need to be accounted for as the temperature increased by 50°C—and Fig. 4 predicts, conversely, that the $\Delta ASA$ of binding will decrease with increasing temperature. Clearly the correlation of $\Delta ASA$ and $\Delta Cp$ is completely inapplicable to the binding of Taq/Klentaq to DNA. The collective analysis of Fig. 2 suggests that such inapplicability of any direct $\Delta ASA$-$\Delta Cp$ correlation may also extend to more than half of all protein-DNA interactions.

It should be clarified that these analyses do not contradict the longstanding and well-established relationship between the sign of $\Delta Cp$ and the burial of polar versus nonpolar surface area (the $\Delta Cp$-hydrophobic effect correlation). What these analyses do suggest, however, is that quantitative $\Delta Cp$-$\Delta ASA$ relationships for protein-DNA interactions may be seriously perturbed by what may be a natural prevalence of temperature-dependent heat capacity changes. I.e., if the results of Figs. 2 and 3 are not merely statistical anomalies, then no current $\Delta ASA$-$\Delta Cp$ correlation can be universally applied to all protein-DNA interactions. Kozlov and Lohman (7) have made a similar argument based on their documentation of both temperature and anion dependencies of $\Delta Cp$ values for the *E. coli* SSB-DNA binding interaction. It may be possible, with adequate additional data, to add correction factors to these relationships, but this begs the question of how far one should stretch/adapt this correlation to attempt to fit all protein-DNA binding data. In our prior study of $\Delta Cp$ effects for Taq/ Klentaq and Klenow polymerases, we suggested that DNA-binding interactions can be sorted into two bins: those with and those without a strong $\Delta Cp$-$\Delta ASA$ correlation (14). For those systems where the correlation holds, the binding is likely dominated by the hydrophobic effect, while those systems for which the correlation does not hold must have other major molecular contributions to the binding and thus to their $\Delta Cp$ values.

### *Potential origins of $\Delta\Delta Cp$*

The analysis in this study cannot address the origins of the observed $\Delta\Delta Cp$ values, but the main categories of potential sources can be discussed. It may be that $\Delta H$ versus temperature is inherently nonlinear for protein-DNA interactions. Linked molecular processes can also explain a temperature-dependent $\Delta Cp$. The molecular nature of an appropriately linked reaction could include any of a number of processes proposed to exhibit a $\Delta Cp$, including DNA distortion (46,48), restriction of vibrational freedom (23,35,49), linked protonation/deprotonation (50,51), multiple cooperative weak interactions (52), and, of course, additionally linked changes in surface area exposure (such as coupled folding-unfolding) (3,31,38–42).

Linked equilibria can only explain the observed $\Delta\Delta Cp$ pattern if there exists a very specific combination of two partially overlapping enthalpic events. For two linked reactions to produce a concave-down curved $\Delta H$ versus temperature dependence (as found for 26 of the data sets examined herein) the following must be true: 1), the two processes must have differing $\Delta Cp$ values; 2), the two processes must have different temperature ranges; and 3), both processes must have negative $\Delta Cp$ values. If any one of these is not true, the observed curvature will not result: 1), if both processes have the same $\Delta Cp$, there is no change in slope of $\Delta H$ versus temperature; 2), if both processes have exactly overlapping temperature ranges, a cumulative $\Delta Cp$ will be observed, but no curvature; and 3), if one process has as positive $\Delta Cp$ or no $\Delta Cp$, the curve will be concave-up or will plateau.

Recent studies of heat capacity effects in protein-protein interactions have quantitatively accounted for some amount of similar concave-down curvature in plots of $\Delta H_{\text{binding}}$ versus temperature by including a term for the temperature-dependent fractional contribution of the unfolding enthalpy (53,54). While in the preceding section we briefly discussed potential contributions of coupled folding/unfolding to the magnitude *of* $\Delta Cp$, these recent studies explore the potential for contributions of folding/unfolding to the presence of a $\Delta\Delta Cp$. Even small amounts of unfolding (~1%) in the experimental binding range can result in visible curvature of $\Delta H_{\text{binding}}$ versus $T$ (53). The typically much larger magnitude of $\Delta H_{\text{folding}}$ versus $\Delta H_{\text{binding}}$ is what makes this possible. A similar analysis of our Taq/Klentaq data (Fig. 1, top, analyzed with Eq. 7 from (54)) indicate that these proteins would only need to unfold (and then refold upon binding) by 8% across the binding temperature range (10–60°C) to account for the experimental curvature in this data. However, previous thermal denaturation studies on Taq and Klentaq from our laboratory clearly show that neither protein even begins to unfold ($\ll$1%) before 85°C (43). This reinforces the conclusion further that coupled folding-unfolding does not significantly contribute to $\Delta Cp$ or $\Delta\Delta Cp$ of DNA binding by Taq/Klentaq. It is certainly possible, however, that such coupled unfolding/refolding may account for some of the

$\Delta\Delta Cp$ values observed in other protein-DNA systems in Table 1. Given the significant consequences that even very small $\Delta\Delta Cp$ values have on the determination of $\Delta Cp$, and for any quantitative predictive application of heat capacity information, continued investigation of this effect seems warranted.

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