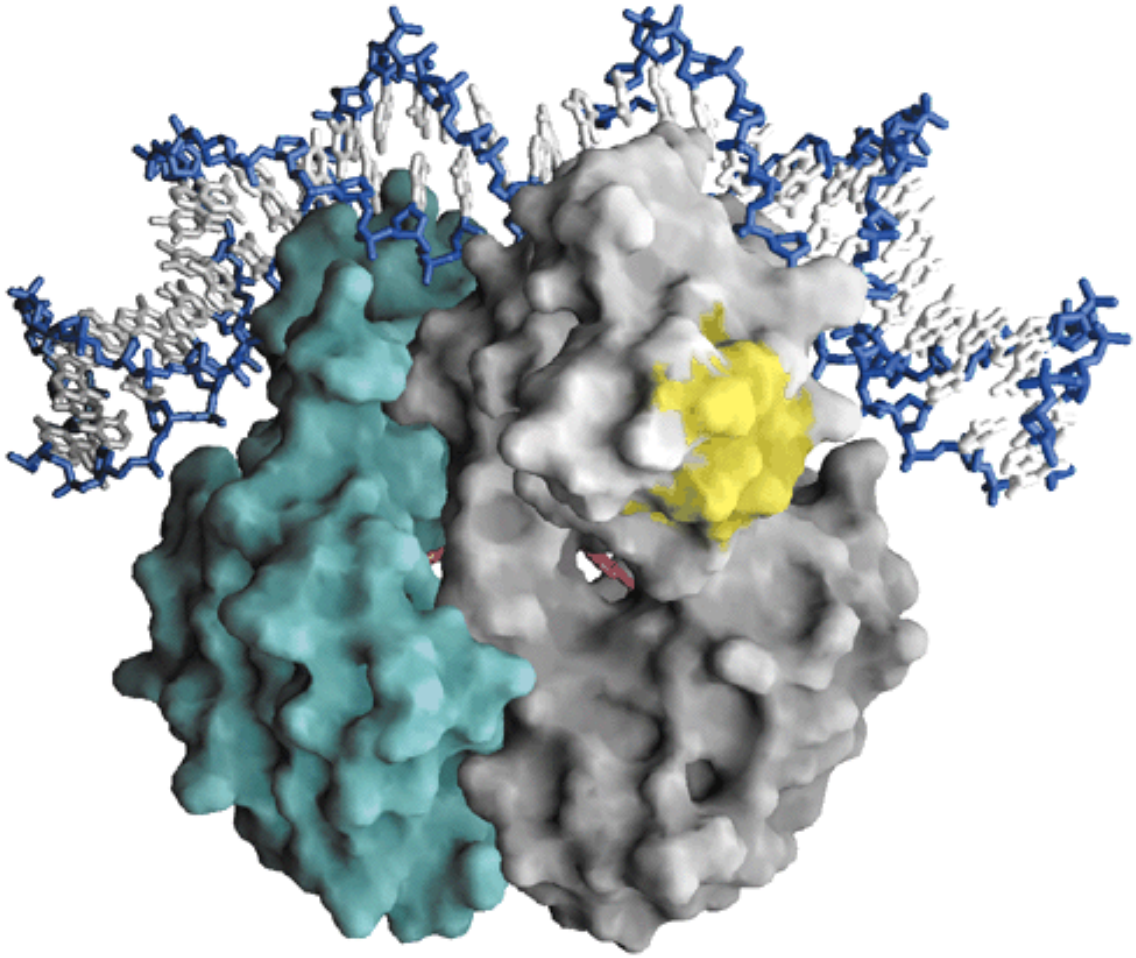
Enthalpy and entropy of protein binding to DNA



The mass equation law for binding of a protein P to its DNA

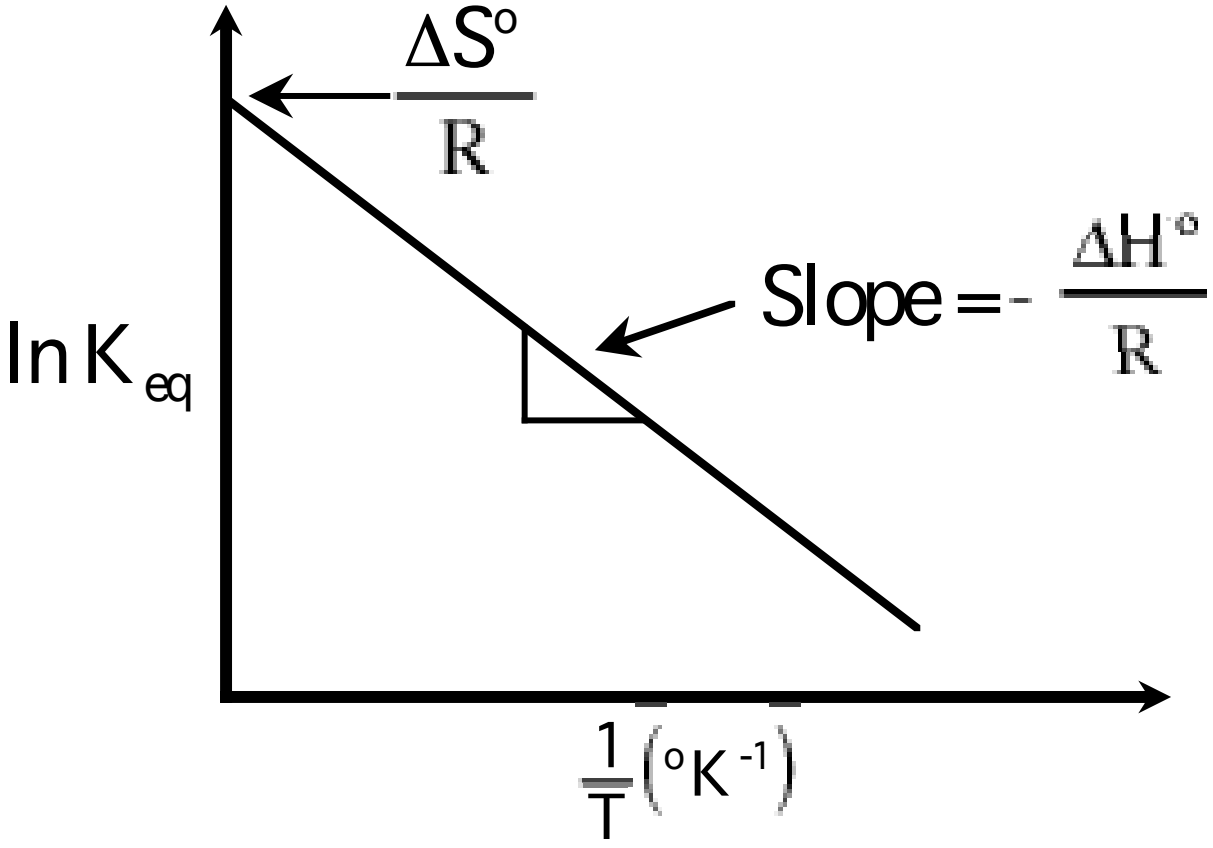
$$D_{\text{free}} + P_{\text{free}} \rightleftarrows DP \qquad K_1 = \frac{D_{\text{free}} \cdot P_{\text{free}}}{DP}$$

binding of the first proteins with the dissociation constant K_1

D_{free}, concentration free DNA; P_{free}, concentration free protein

$$\text{binding constant } K_B = \frac{1}{\text{dissociation constant } K_D}$$

Temperature dependence of the binding constants reveals ΔH and ΔS (van't Hoff plot)



$$\Delta G = \Delta H - T \cdot \Delta S$$

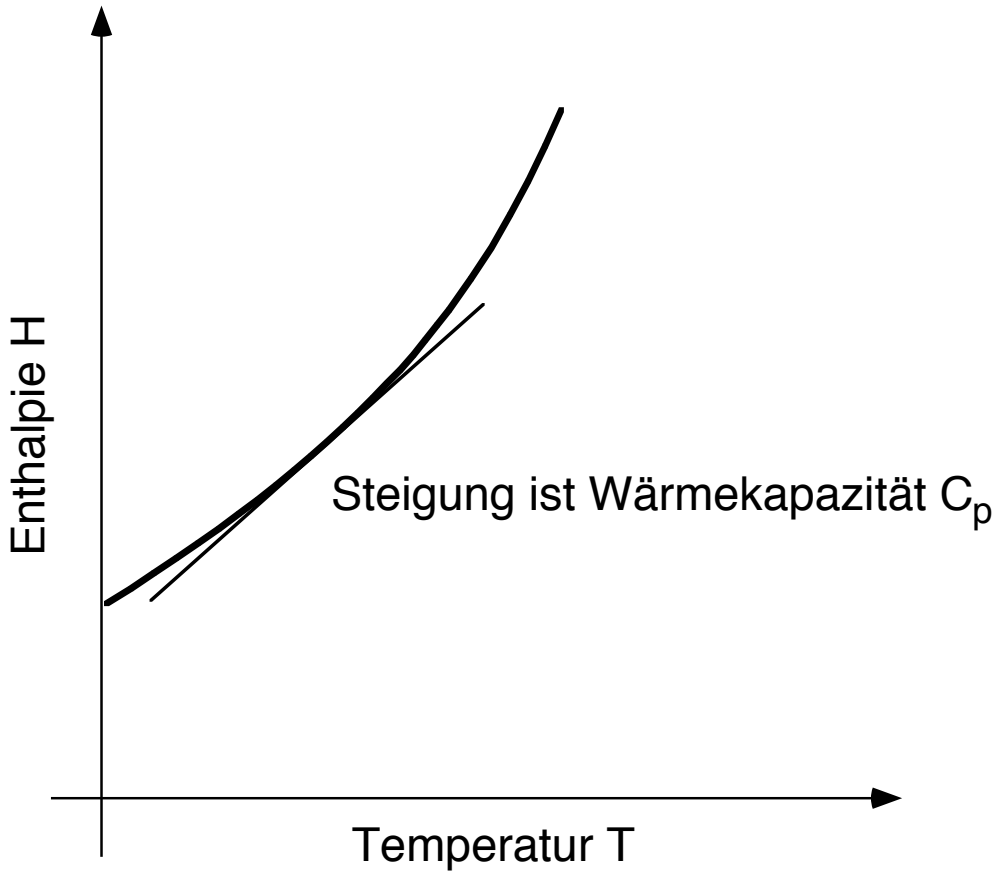
$$\Delta G = -R \cdot T \cdot \ln K_{eq}$$

$$\ln K_{eq} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

$$\frac{\partial(\ln K_{eq})}{\partial(1/T)} = -\frac{\Delta H}{R}$$

From the slope of ln K_{eq} vs. 1/T (usually from 0 to 40 °C) one can determine the ΔH and from extrapolation also ΔS . Is the van't Hoff plot curved then ΔH is temperature dependent.

The heat capacity C_P in JK is the amount of heat Q to produce a unit change in temperature T



C_P describes the temperature dependence of ΔH and ΔS

C_P is assumed to be constant (good approximation for the narrow interval from 0 to 40 °C)

$$C_{\mathrm{P}} = \frac{Q}{\Delta T}$$

$$H(T_2) - H(T_1) = C_{\mathrm{P}}(T_2 - T_1)$$

$$S(T_2) - S(T_1) = C_{\mathrm{P}} \ln\left(\frac{T_2}{T_1}\right)$$

Relation between ΔC_P, ΔG and K_{eq} for binding

For two characteristic temperature T_H and T_S with

$$\Delta H(T_H)=0 \text{ and } \Delta S(T_S)=0 \quad \Rightarrow$$

$$\Delta H(T)=\Delta C_P\cdot(T-T_H)$$

$$\Delta S(T)=\Delta C_P\cdot\ln\left(\frac{T}{T_S}\right)$$

$$\Delta G(T)=\Delta C_P\cdot(T-T_H)-T\cdot\Delta C_P\cdot\ln\left(\frac{T}{T_S}\right)$$

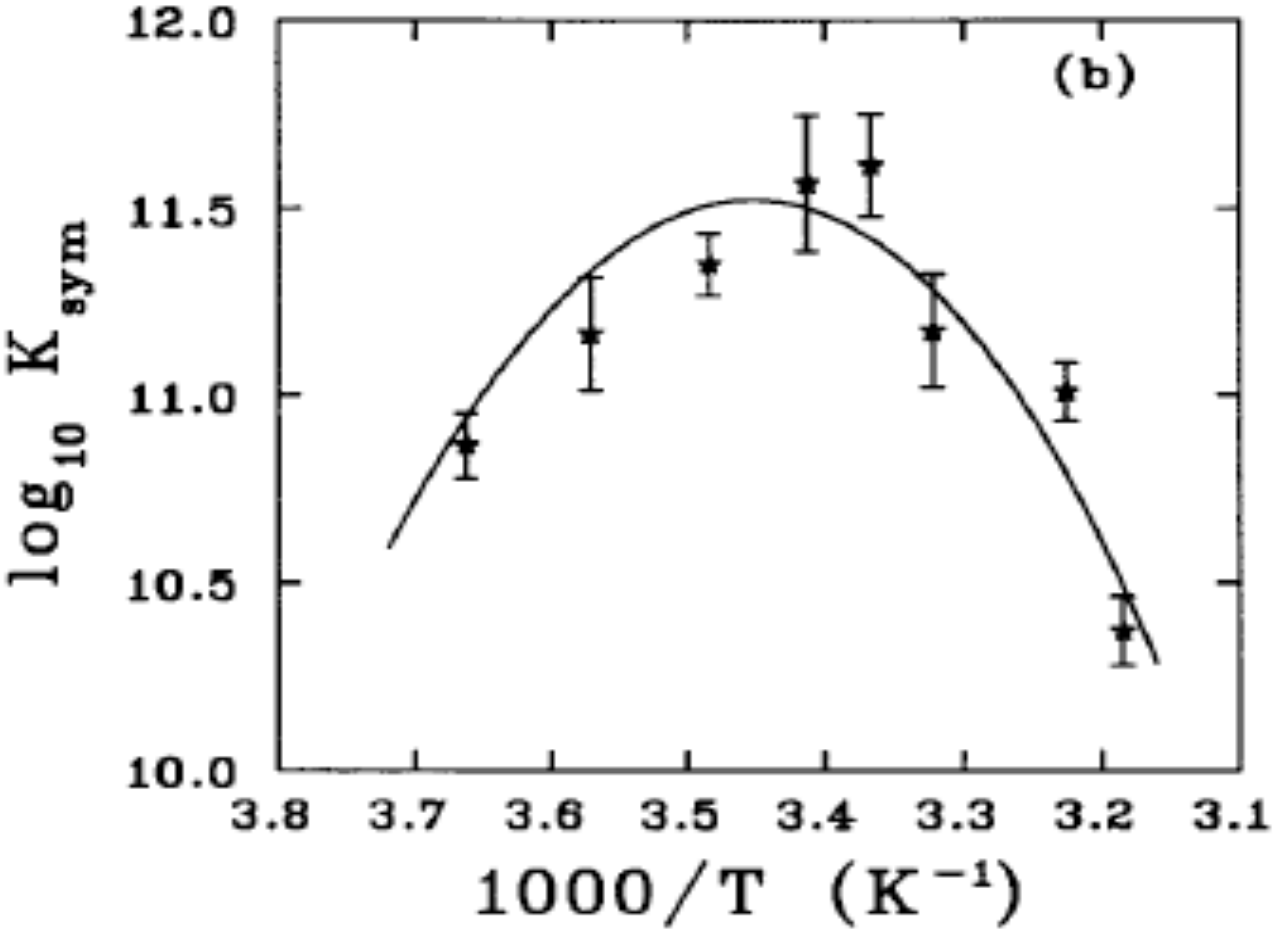
$$\Leftrightarrow$$

$$\ln K_{eq}=\frac{\Delta C_P}{R}\cdot\left[\frac{T_H}{T}-1-\ln\left(\frac{T_S}{T}\right)\right] \qquad \ln K_{eq}=-\frac{\Delta H}{RT}+\frac{\Delta S}{R}$$

Temperature dependent ΔH and ΔS.

van't Hoff plot

Temperature dependence of equilibrium binding constant for specific binding of lac repressor to the operator DNA



Relationship between heat capacity Cp and non-polar surface of amino acids

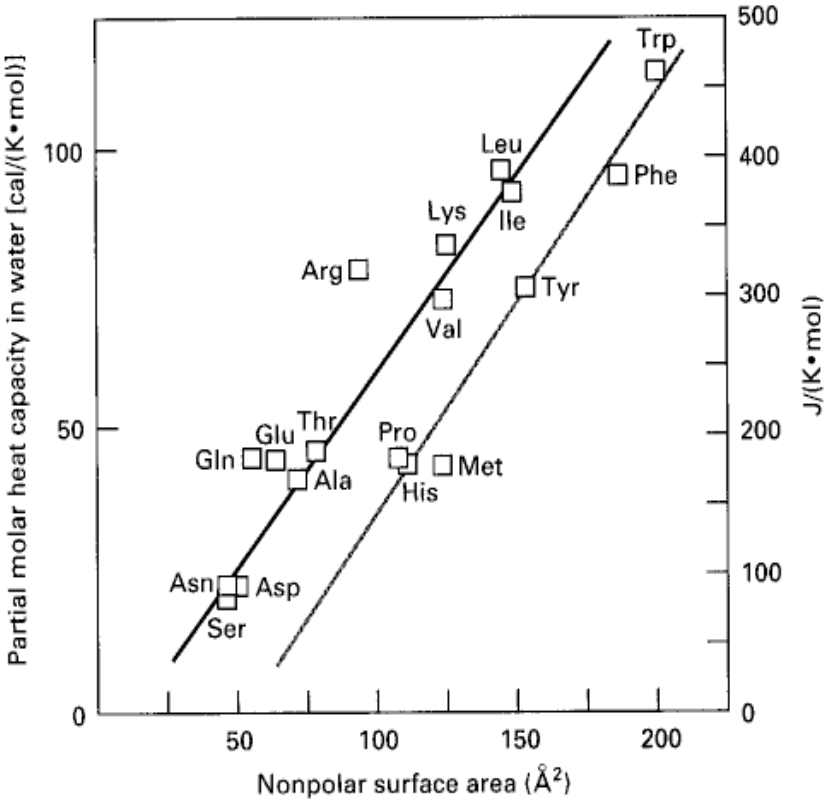


FIGURE 4.13
Correlation between the heat capacities in aqueous solution at 25°C with the accessible surface area of the nonpolar atoms of analogues of the amino acid side chains. The upper straight line fits all the side chains except those with ring structures and the sulfur-containing Met (lower line). The slope of the upper line is 0.72 cal/K·mol Å² (300 J/K·mol nm²). (Adapted from G. I. Makhatadze and P. L. Privalov, *J. Mol. Biol.* 213:375–384, 1990.)

- Cp proportional to the non-polar surface area
- Hydrophobic effect: ordered water structure around non-polar amino acids
- Large Cp is “hallmark” of hydrophobic effect

Relationship between heat capacity change ΔCp and non-polar surface area for protein folding

- ΔC_p is correlated with non polar surface area ΔA_{np}

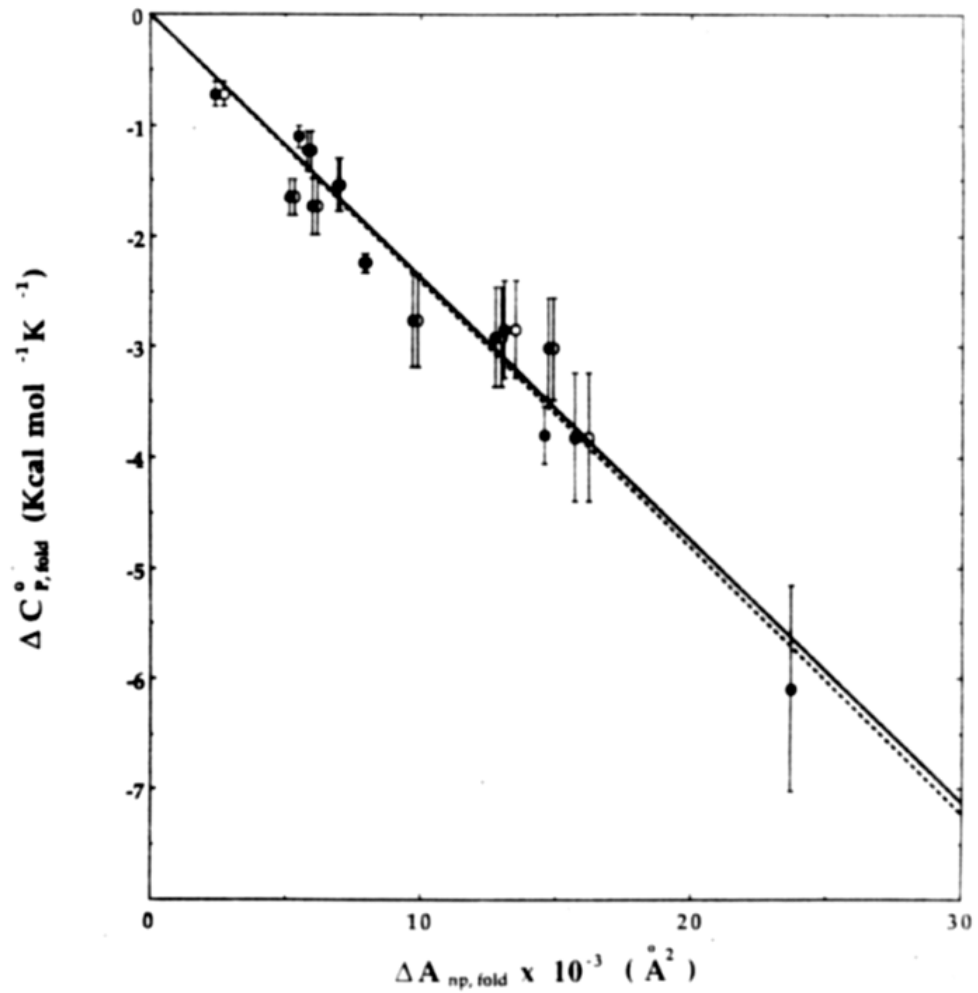
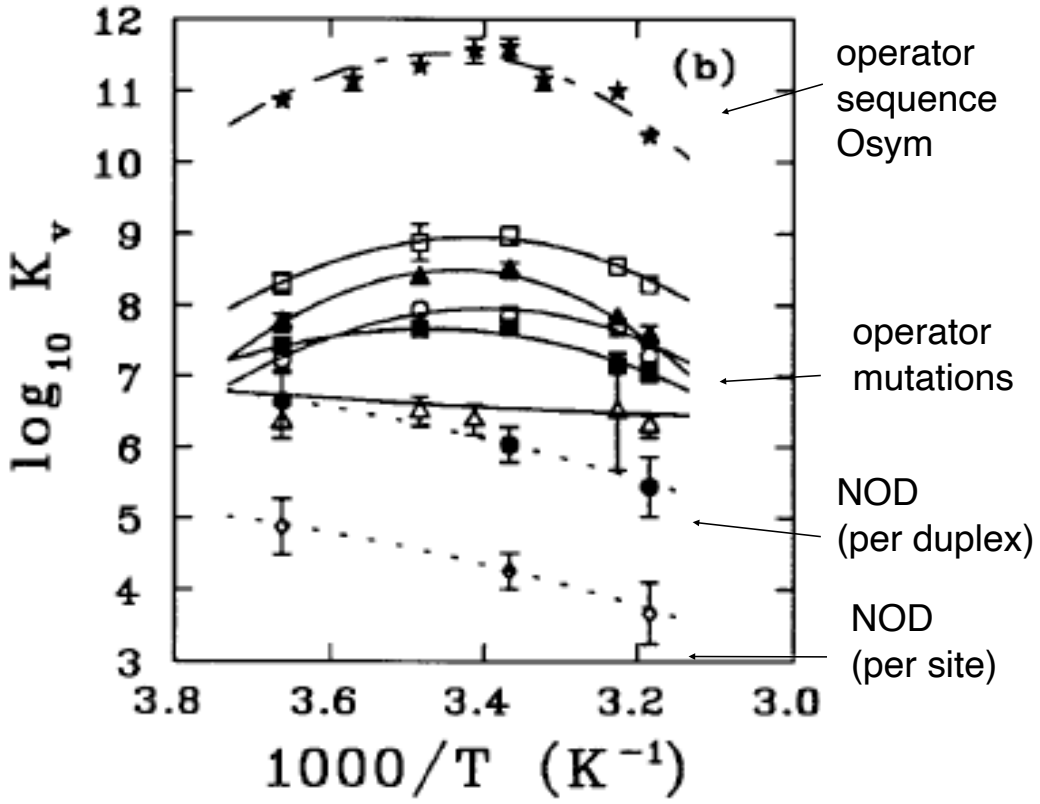


FIGURE 3: Standard heat capacity changes ($\Delta C^{\circ}_{p,fold}$) for the process of protein folding as a function of the reduction in water-accessible nonpolar surface area accompanying folding (ΔA_{np}). The denatured state is assumed to be in the extended β-form. The solid line is the weighted least-squares fit obtained by using set 1 radii (○) to calculate ΔA_{np}; the dashed line is the fit obtained by using set 2 radii (●). Where the two values of ΔA_{np} agree within the size of the data point, only one point (●) is plotted.

From Livingstone JR, Spolar RS, Record MT Jr. Biochemistry. 1991 Apr 30;30(17):4237-44

Temperature dependence of Kd for specific/nonspecific binding of lac repressor => less induced folding in the unspecific complex

specific binding vs.
unspecific binding



O^{sym} Fragment:

10 9 8 7 6 5 4 3 2 1

5' GTAGTGGCGAAATTGTGAGCGCTCACAATTCGTTTGGCCG 3'

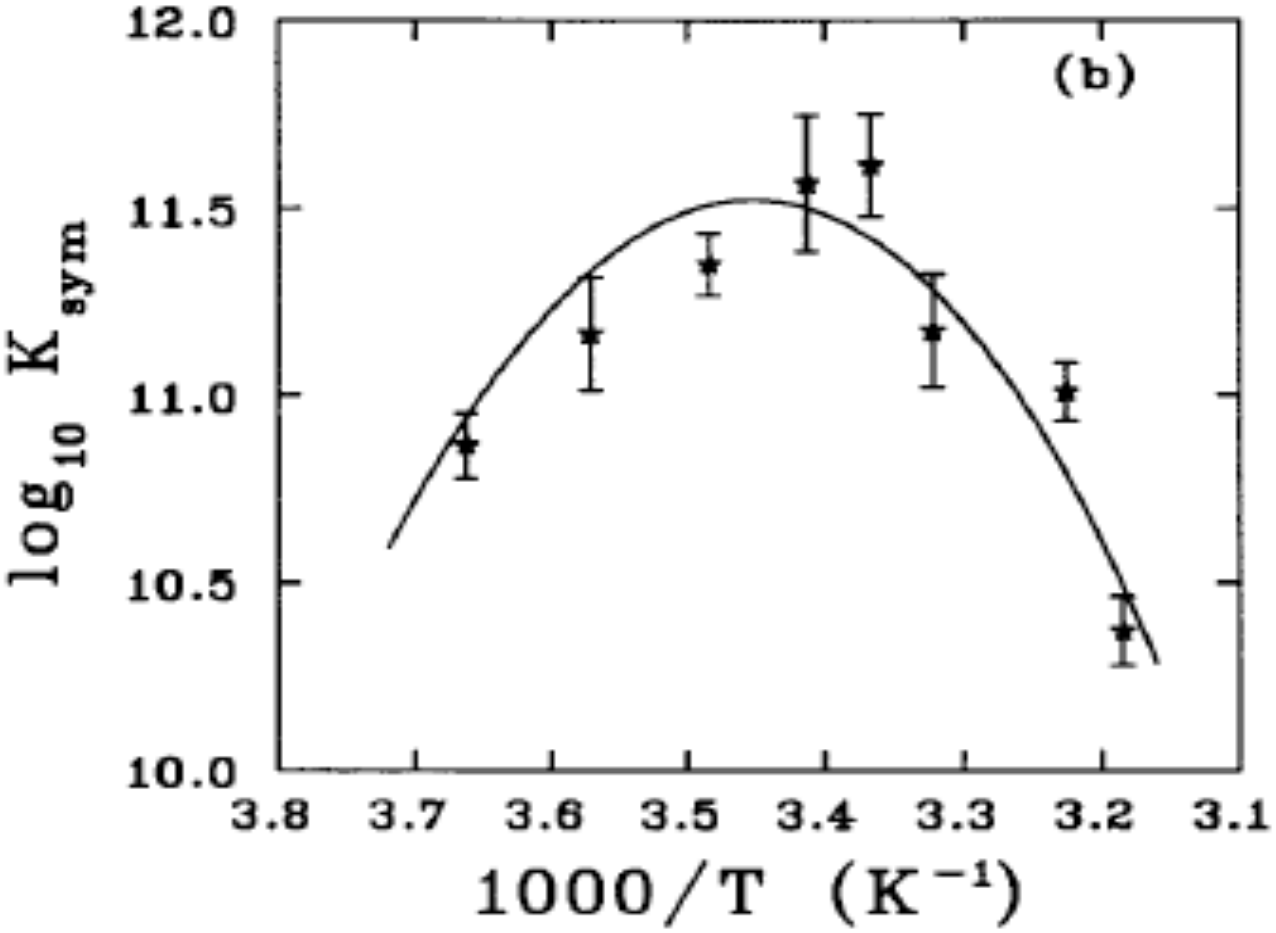
Variant Operators:

O^{4A}	AATTGTAAGCGCTTACAATT
O^{5A}	AATTGAGAGCGCTCTCAATT
O^{4A5A}	AATTGAAAGCGCTTTCAATT
O^{5C}	AATTGCGAGCGCTCGCAATT
O^{4A5C}	AATTGCAAGCGCTTGCAATT

Nonoperator Fragment:

NOD	TCTAAGAGTTACTCTATCCG

Temperature dependence of equilibrium binding constant for specific binding of lac repressor to the operator DNA



Temperature dependce of ΔH, ΔS and ΔG for lac repressor binding

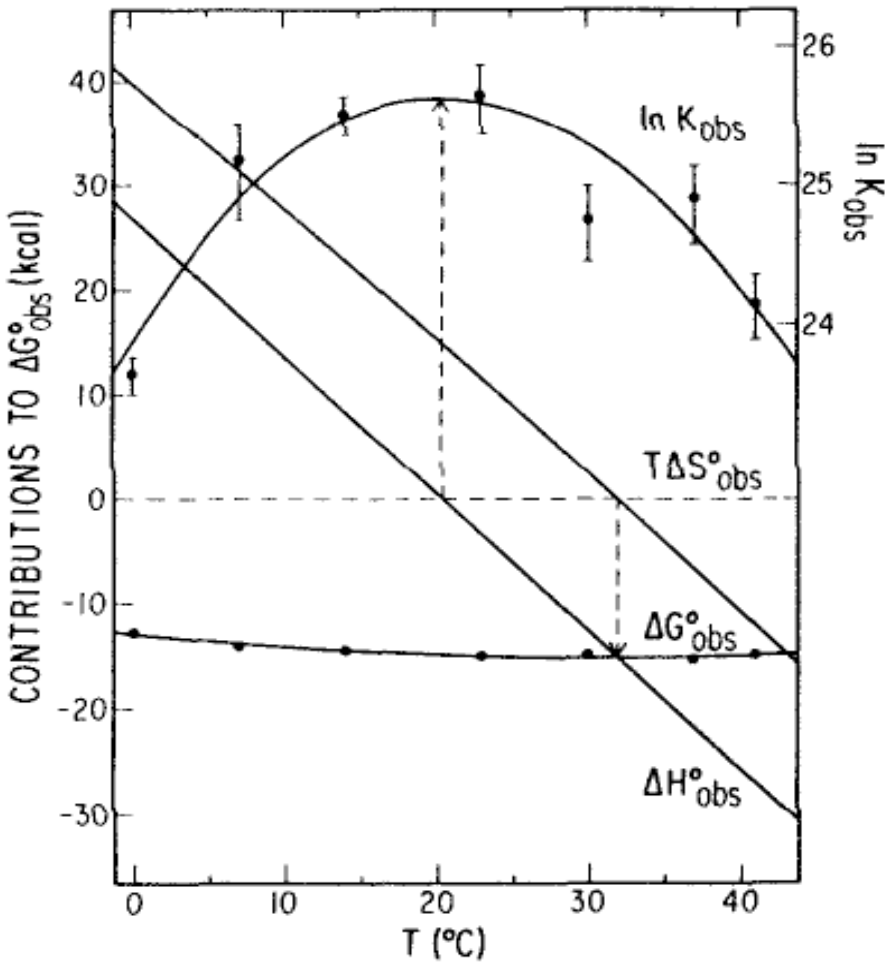


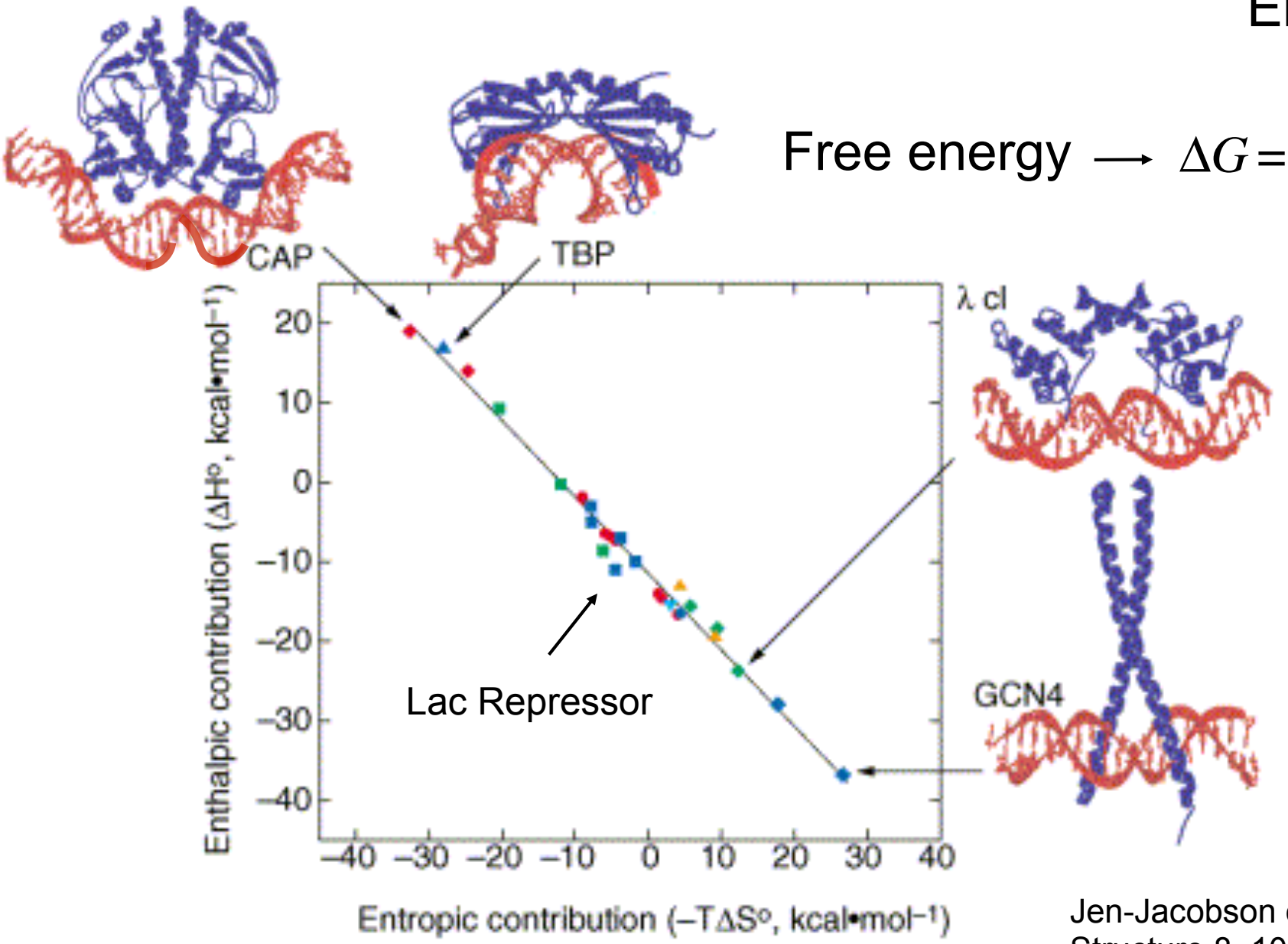
FIG. 2. The thermodynamics of the interaction of *lac* repressor with an isolated symmetric operator (O^{sym}) site. Values of ln K_{obs} and ΔG°_{obs} are plotted as a function of temperature. Enthalpic (ΔH°_{obs}) and entropic ($T\Delta S^{\circ}_{obs}$) contributions to ΔG°_{obs}, as well as theoretical fits to ln K_{obs} and ΔG°_{obs}, were obtained assuming a constant $\Delta C^{\circ}_{P,obs}$ of -1.3 kcal mol^{-1} K^{-1} over the temperature range investigated. [From J.-H. Ha, R. S. Spolar, and M. T. Record, Jr., *J. Mol. Biol.* **209**, 801 (1989).]

The unfavorable enthalpy contribution associated with DNA distortion is compensated by a favorable entropy

Enthalpy

Free energy $\longrightarrow$ $\Delta G = \Delta \mathrm{H} - T \cdot \Delta S$

Entropy



Jen-Jacobson et al. (2000). Structure 8, 1015-1023

K_D and ΔG values for protein-DNA binding per site

Specific binding of a protein to DNA varies over a relatively small range of $\Delta G_{bind,sp}$ = -9 to -16 kcal/mol, with ~60 kcal/mol for ΔH and $T\Delta S$

$\Rightarrow \Delta G_{bind,sp} \approx$ const. (-11.7 ± 1.6 kcal/mol)

$\Rightarrow \Delta H = -T \cdot \Delta S$ - 11.7 kcal/mol

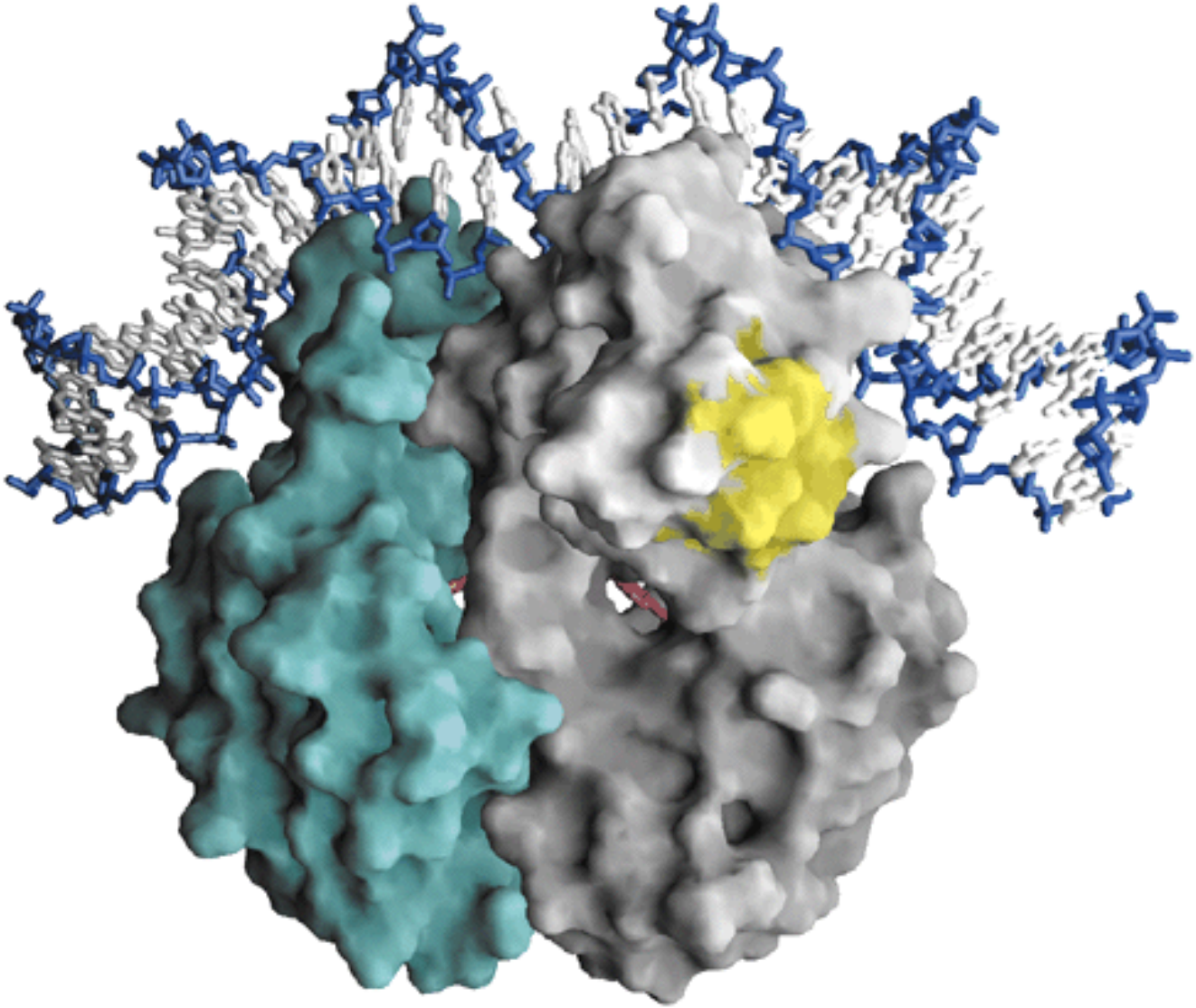
Protein needs to select specific binding site from unspecific sites

$\Rightarrow \Delta\Delta G$(specific - unspecific) ~ -5 to -9 kcal/mol

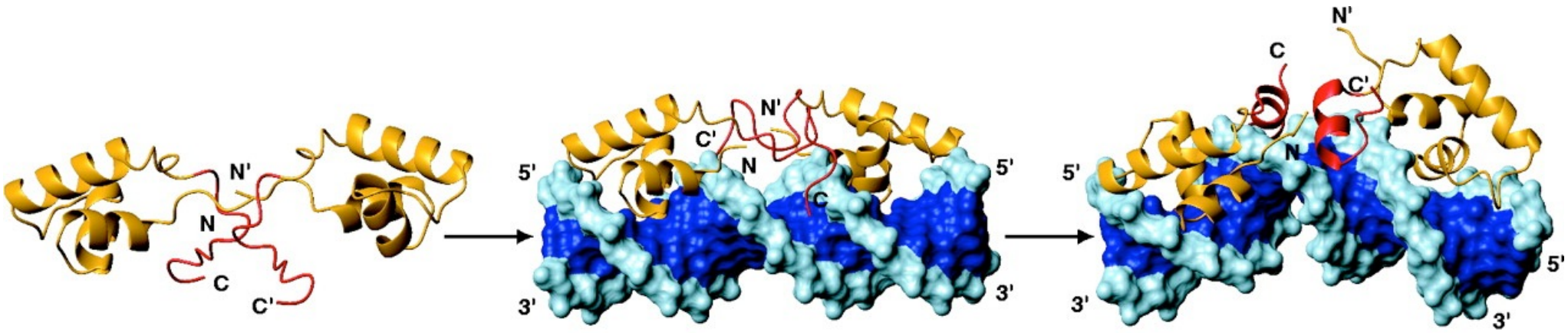
Protein binding must be reversible on the cell's time scale

$\Rightarrow \Delta G_{bind,sp} \leq$ -16 kcal/mol

Molecular structure of E. coli CRP (also called CAP for catabolite gene activator protein)

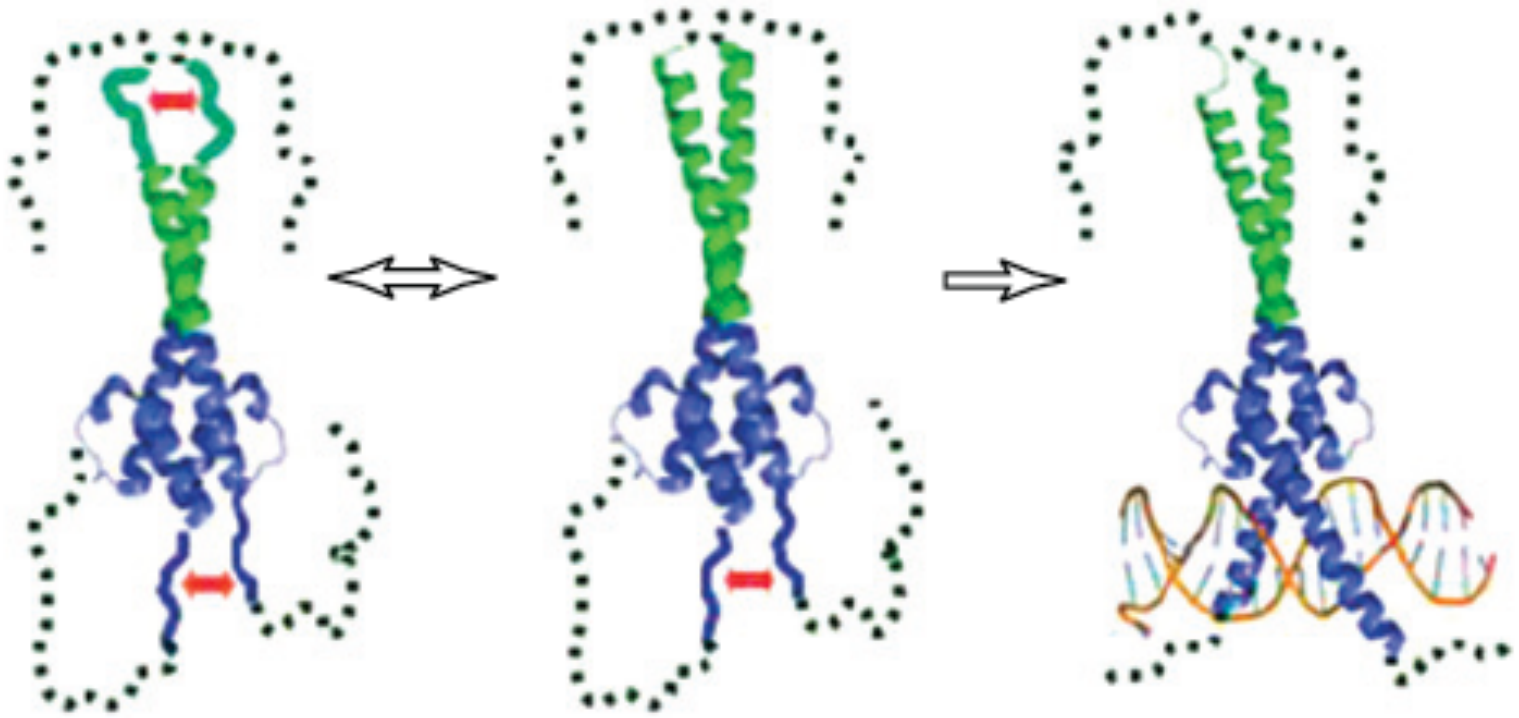


The hinge region (50-62 in red) of Lac-DBD is folded only in the specific complex with DNA



- folding of hinge region with specific contacts in minor groove
- specific interactions major groove
- less electrostatic interactions
- curvature of DNA

Local folding of the Max transcription factor upon dimerization and binding



The Max transcription factor (PDBcode: 1NKP) binds DNA as a dimer. The disordered N-terminal region (upper dotted line) reduces the electrostatic repulsion (red arrows) between the two monomers, and increases the population of the folded state at the flanking leucine zipper (green). This also stabilizes the bHLH region (blue) and thus improves binding affinity for DNA.

Application from temperature dependence of ΔH and ΔS to specific protein-DNA binding

- A large negative heat capacity is observed
- This suggests burial of nonpolar surface area
- In addition folding/conformational changes of the protein occur upon DNA binding
- For specific/unspecific binding this effect can be different
- Example: lac repressor