Problem set notes

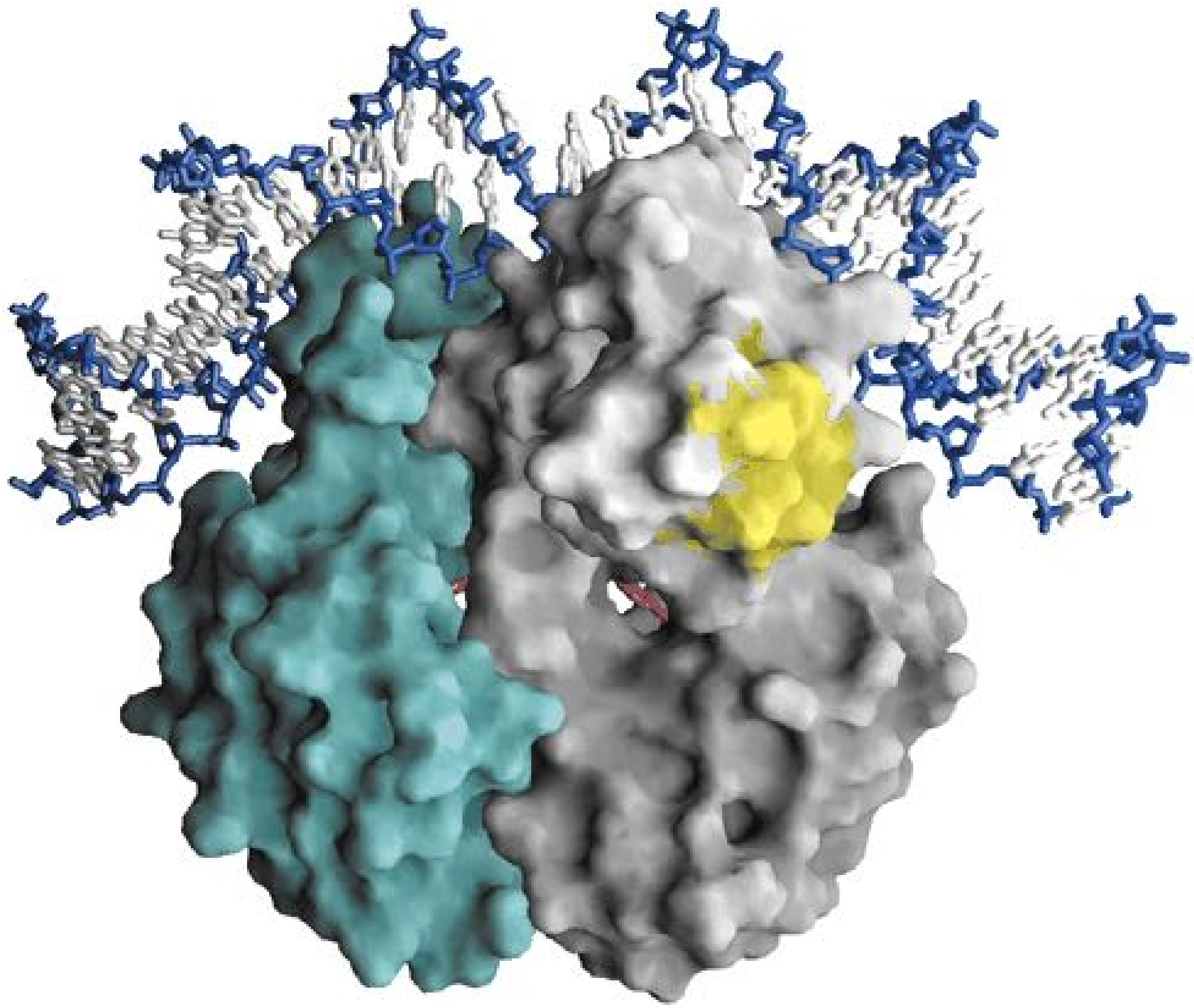
- I have received 33 problem sets so you can multiply every step by 33…
- Include [BPC2025] in the subject line of your e-mail so that I find it.
- If you have not received a reply e-mail from me for the first problem set, drop me a note and I search for it.
- Name your problem set as "*ProblemNumber_FirstName_LastName*", e.g., 01_Karsten_Rippe to make my life easier.
- Keep the file size of your problem to be below 6 MB and 6 pages.
- If you have rasterized images or scanned/imaged hand written notes convert them to jpeg files with appropriate resolution and reduction so that a DIN A4 page is not larger than 1 MB.

Image processing and figure making

https://www.affinity.studio

***** No course next week, Tuesday, Nov 11 notes *****

Enthalpy and entropy of protein binding to DNA



ΔG of a reaction in equilibrium

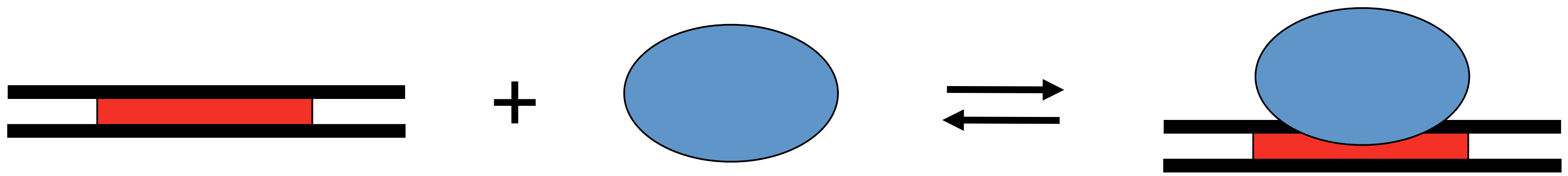
$$\Delta G = \Delta \mathrm{H} - T \cdot \Delta S$$

$$a\mathrm{A} + b\mathrm{B} + \ldots \rightleftarrows g\mathrm{G} + h\mathrm{H} \ldots$$

$$\Delta G^0 = -RT \ln\left(\frac{[\mathrm{G}]^{\mathrm{g}}[\mathrm{H}]^{\mathrm{h}} \ldots}{[\mathrm{A}]^{\mathrm{a}}[\mathrm{B}]^{\mathrm{b}} \ldots}\right)_{\mathrm{Eq}} = -RT \ln K$$

$$K = \left(\frac{[\mathrm{G}]^{\mathrm{g}}[\mathrm{H}]^{\mathrm{h}} \ldots}{[\mathrm{A}]^{\mathrm{a}}[\mathrm{B}]^{\mathrm{b}} \ldots}\right)_{\mathrm{Eq}} = \exp\left(\frac{-\Delta G^0}{RT}\right)$$

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



$$D_{\text{free}} + P_{\text{free}} \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} DP \qquad K_1 = \frac{D_{\text{free}} \cdot P_{\text{free}}}{DP} = \frac{k_{\text{off}}}{k_{\text{on}}}$$

binding of the first proteins with the dissociation constant K_1

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

$$\text{binding constant } K_{\text{B}} = \frac{1}{\text{dissociation constant } K_{\text{D}}}$$

How fast is binding or dissociation

$$AB \underset{k_{\mathrm{on}}}{\overset{k_{\mathrm{off}}}{\rightleftharpoons}} A+B$$

k_{off} in s^{-1} is the reaction rate constant for dissociation

k_{on} in $M^{-1}\ s^{-1}$ is the reaction rate constant for binding

$$\frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} = K_{\mathrm{d}}$$

relation to the equilibrium dissociation constant

$$\frac{1}{k_{\mathrm{off}}} = \tau$$

life time of the complex

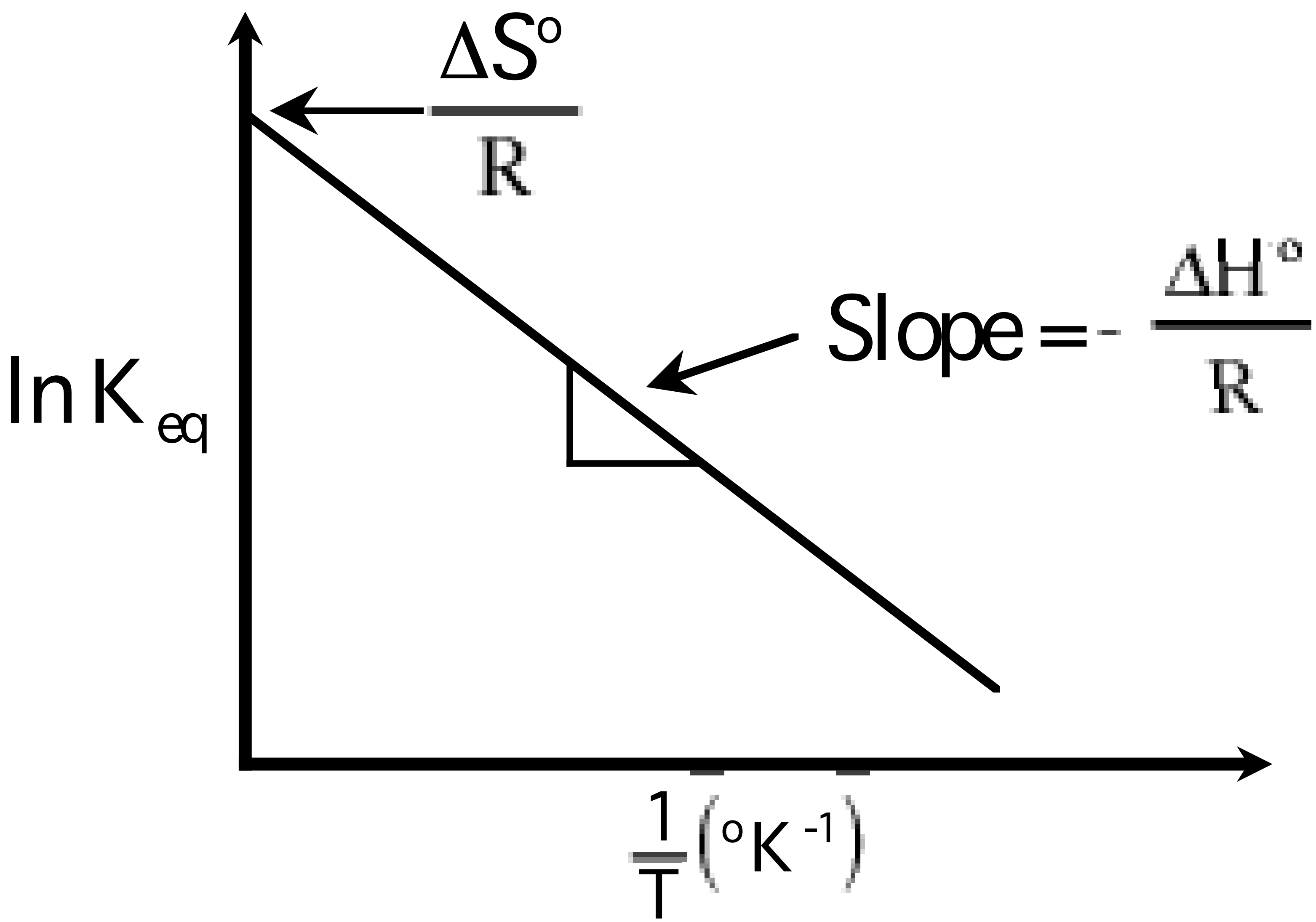
$$\frac{d[\mathrm{AB}]}{dt} = \mathrm{k}_{\mathrm{on}} \cdot [\mathrm{A}] \cdot [\mathrm{B}] - \mathrm{k}_{\mathrm{off}} \cdot [\mathrm{AB}]$$

rate equation for complex formation,

can be solved but it is already difficult

k_{on} cannot be higher than 10^8 - 10^9 $M^{-1}\ s^{-1}$ for a diffusion controlled reaction

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



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

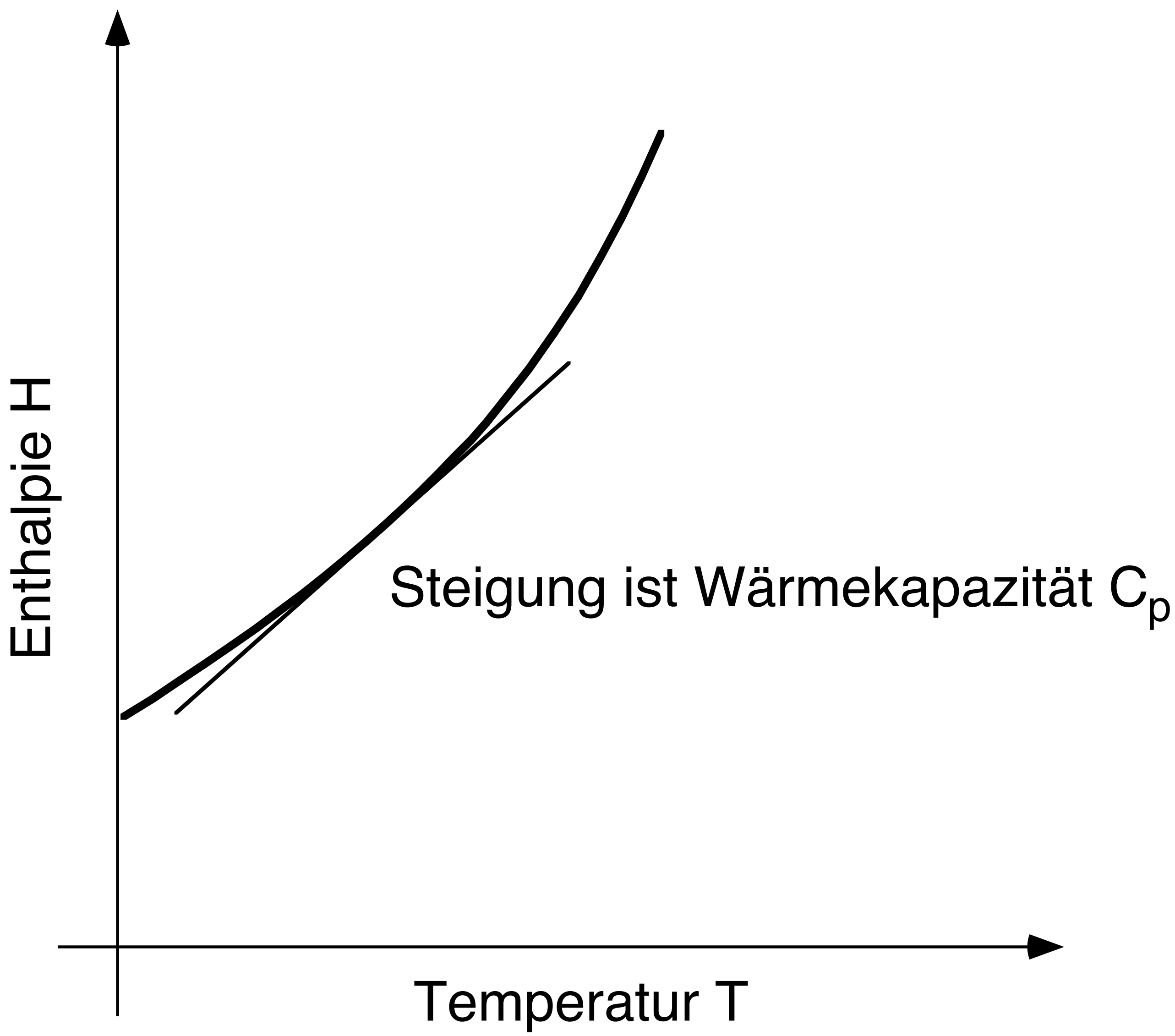
$$\Delta G = -R \cdot T \cdot \ln K_{\mathrm{eq}}$$

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

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

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)

$$C_P = \frac{Q}{\Delta T}$$

$$H(T_2) - H(T_1) = C_P (T_2 - T_1)$$

$$S(T_2) - S(T_1) = C_P \ln\left(\frac{T_2}{T_1}\right)$$

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

For two characteristic temperature T_H and T_S with

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

$$\Delta H(T) = \Delta C_\mathrm{P} \cdot (T - T_H)$$

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

$$\Delta G(T) = \Delta C_\mathrm{P} \cdot (T - T_H) \; - \; T \cdot \Delta C_\mathrm{P} \cdot \ln\left(\frac{T}{T_S}\right)$$

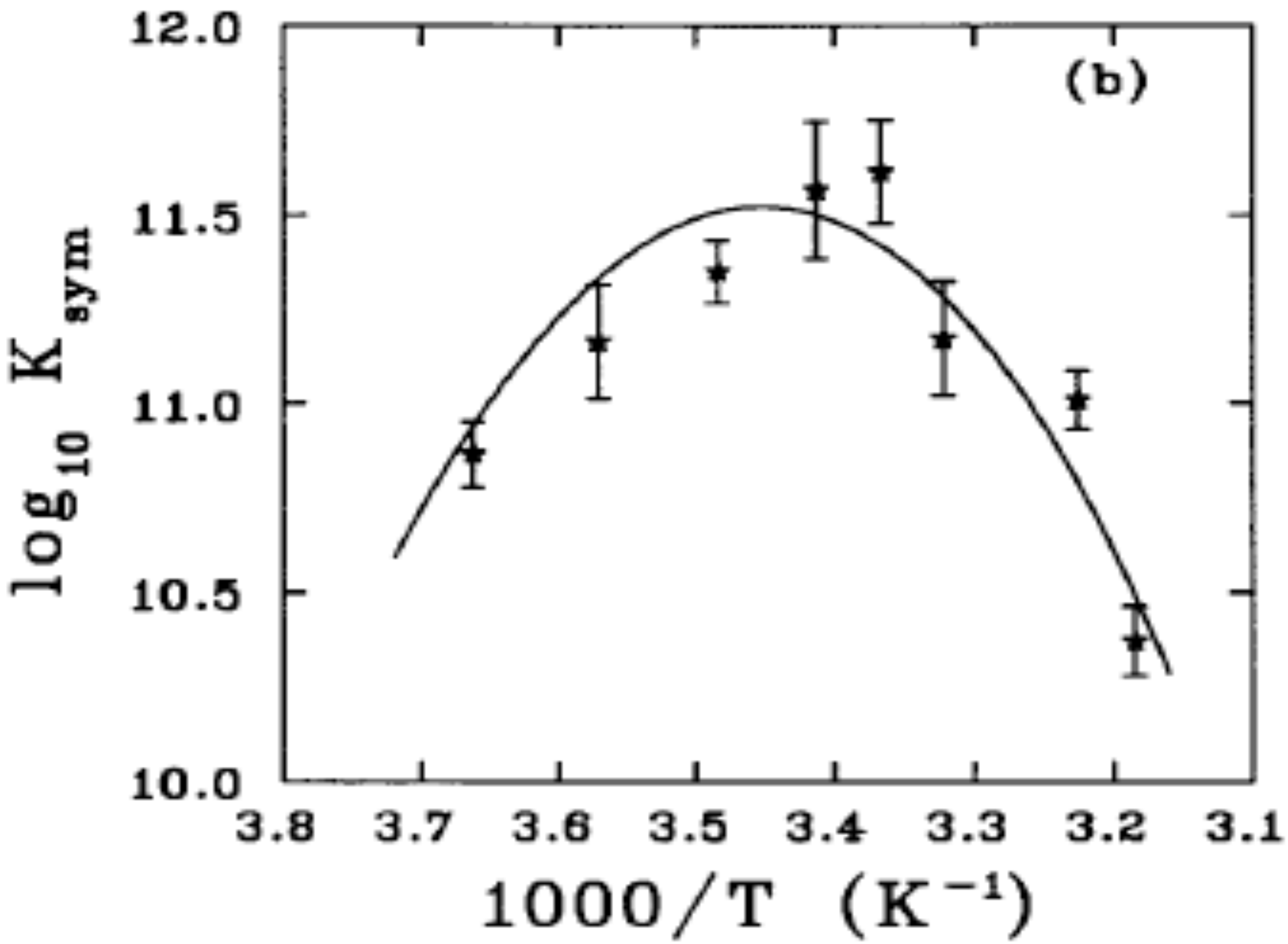
$$\Leftrightarrow$$

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

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

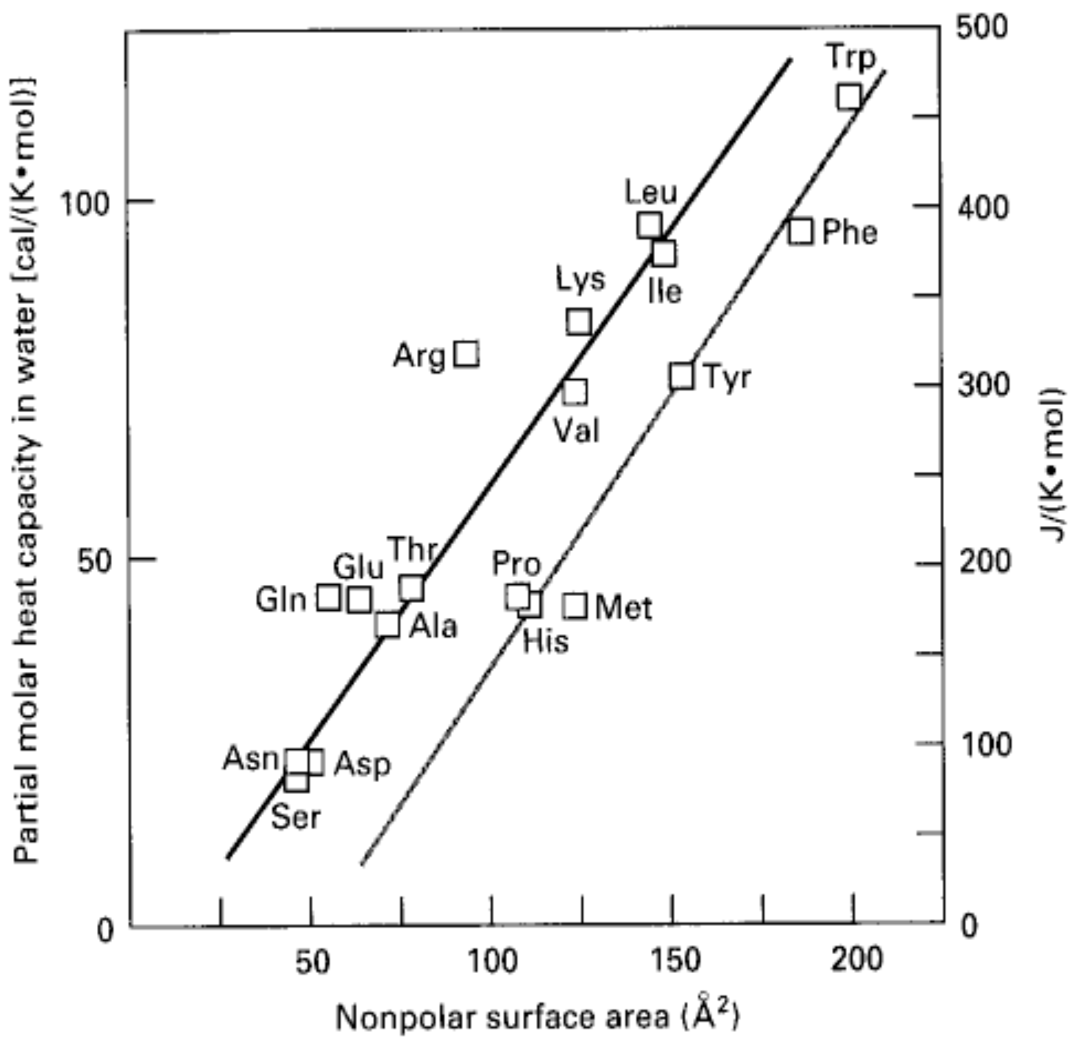


FIGURE 4.13
Correlation between the heat capacities in aqueous solution at 25°C with the accessible surface area of the nonpolar atoms of analogues of the amino acid side chains. The upper straight line fits all the side chains except those with ring structures and the sulfur-containing Met (lower line). 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}

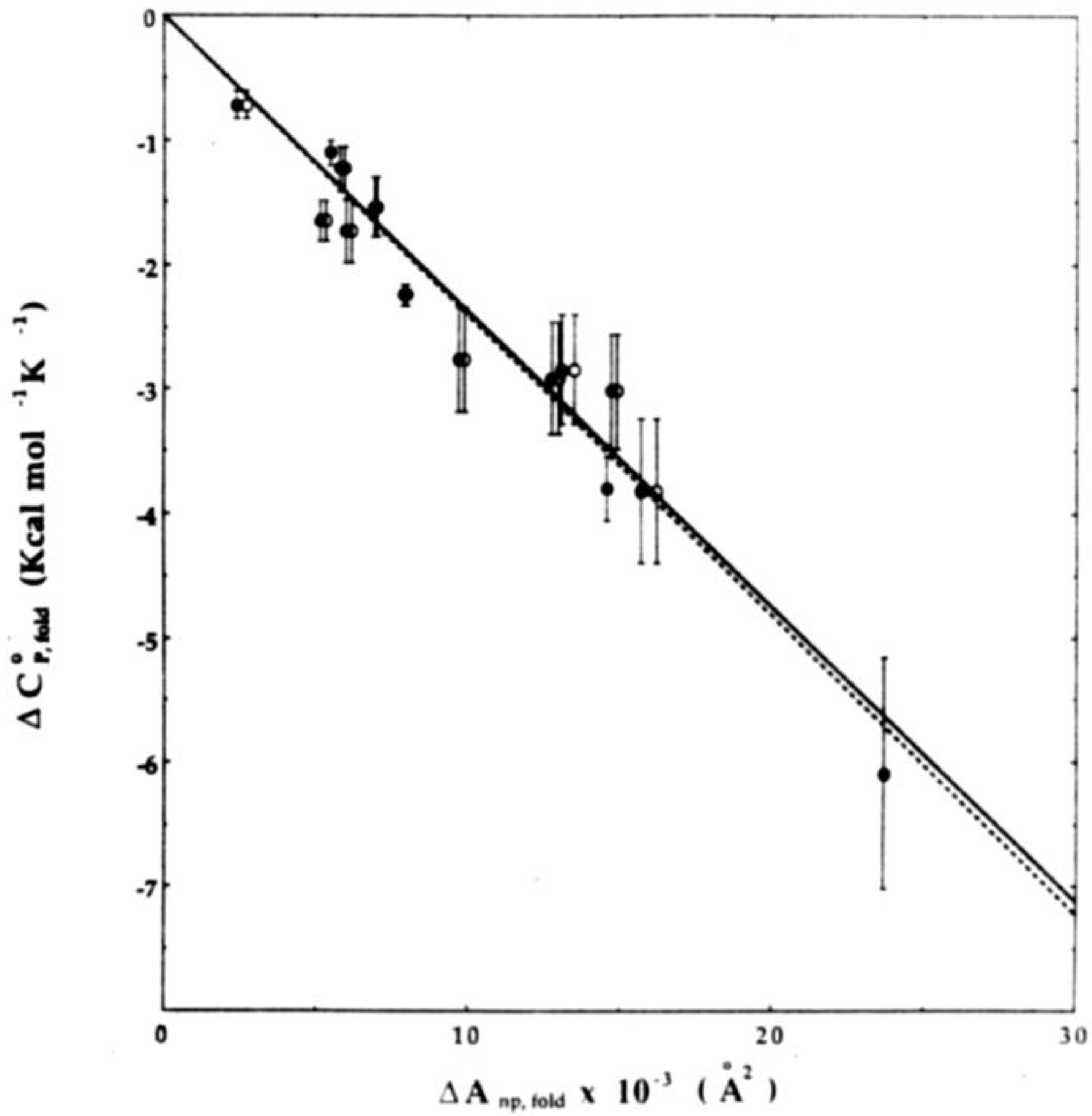


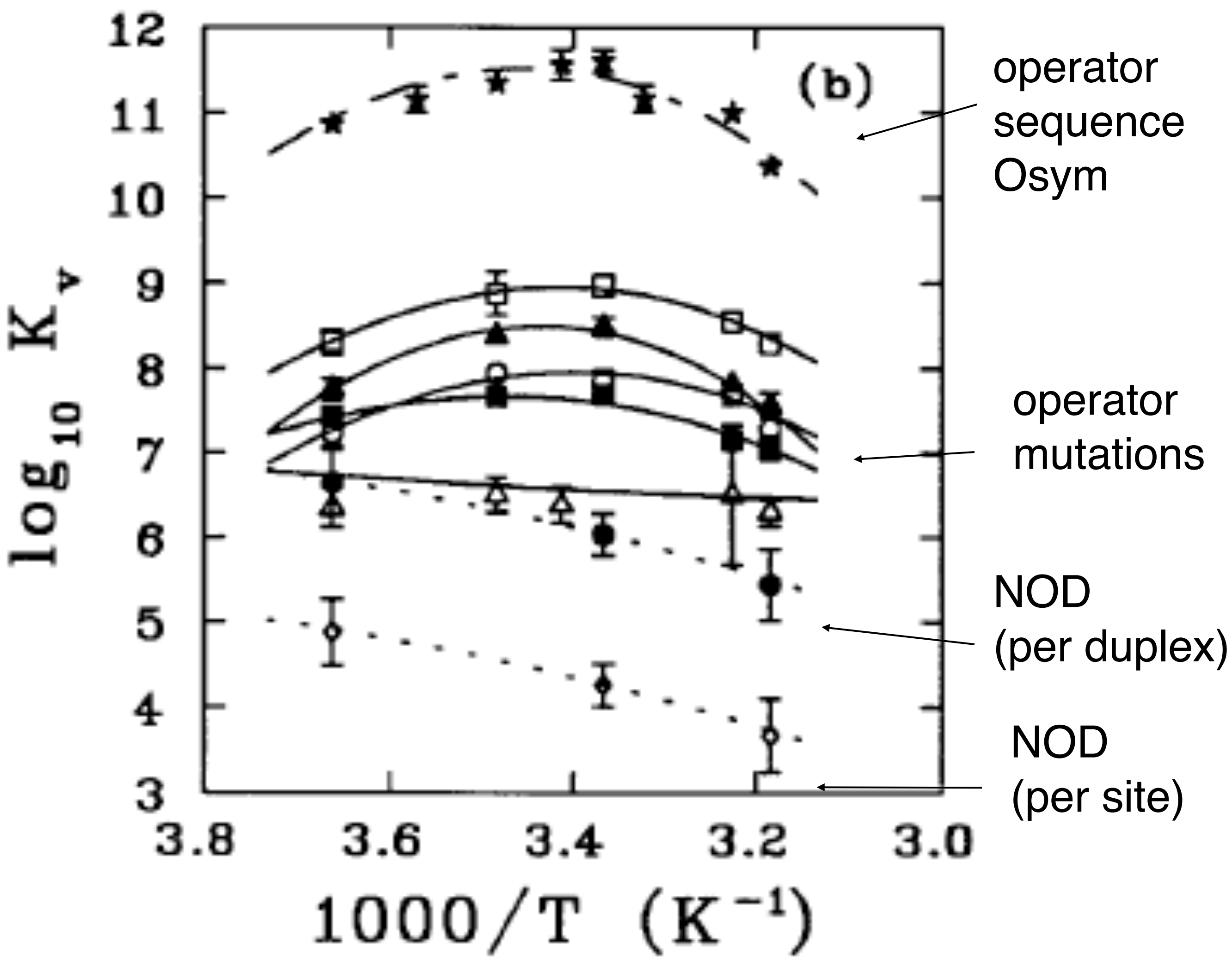
FIGURE 3: Standard heat capacity changes ($\Delta C^\circ_{p,fold}$) for the process of protein folding as a function of the reduction in water-accessible nonpolar surface area accompanying folding (ΔA_{np}). The denatured state is assumed to be in the extended β-form. The solid line is the weighted 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

By measuring the heat capacity change ΔCp between folded and unfolded state or between free protein and DNA bound protein we can detect if non-polar surface are is reduced or increased

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'

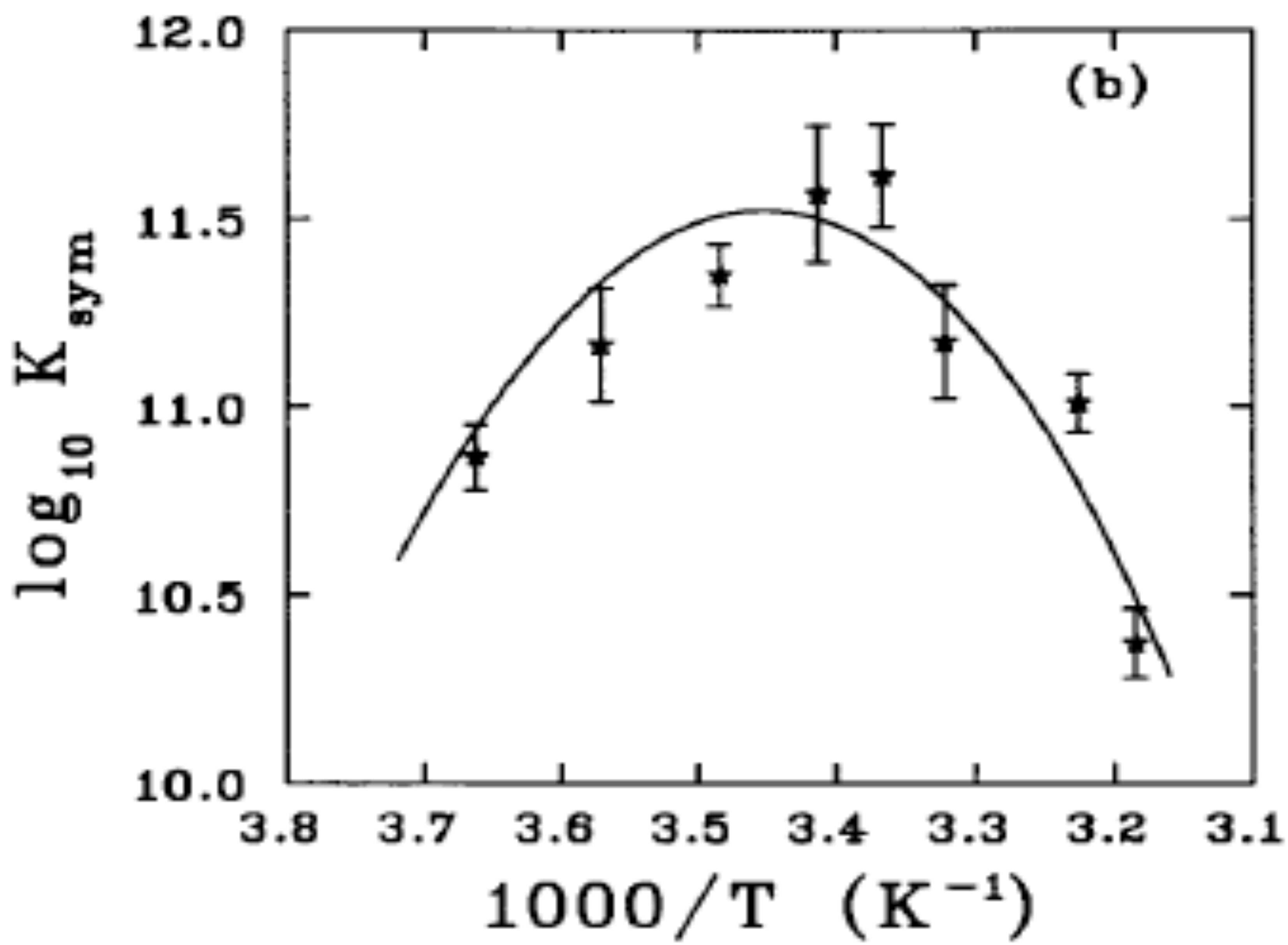
Variant Operators:

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

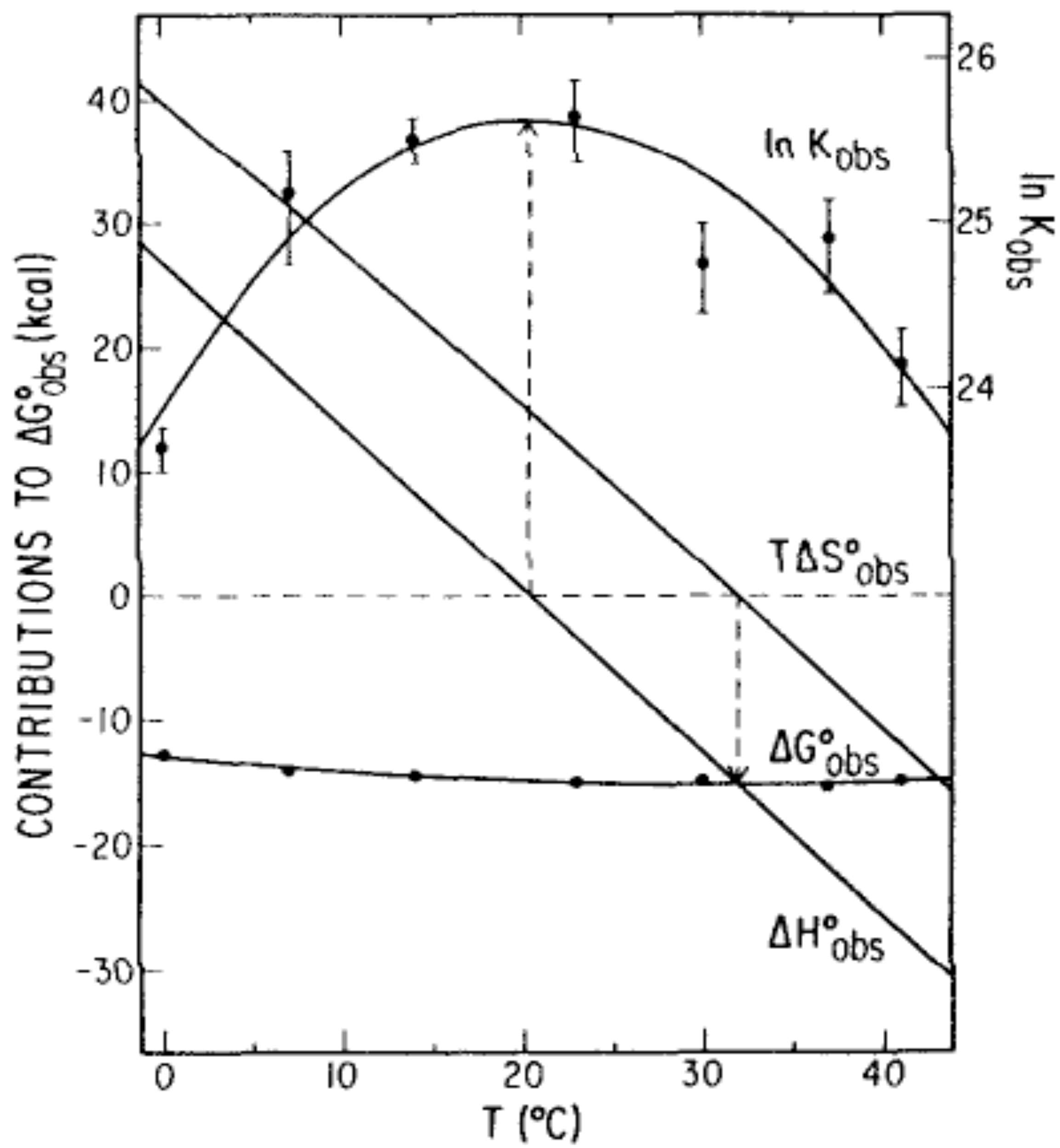


FIG. 2. The thermodynamics of the interaction of *lac* repressor with an isolated symmetric operator (O^{sym}) site. Values of ln K_{obs} and ΔG°_{obs} are plotted as a function of temperature. Enthalpic (ΔH°_{obs}) and entropic ($T\Delta S^\circ_{obs}$) contributions to ΔG°_{obs}, as well as theoretical 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).]

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