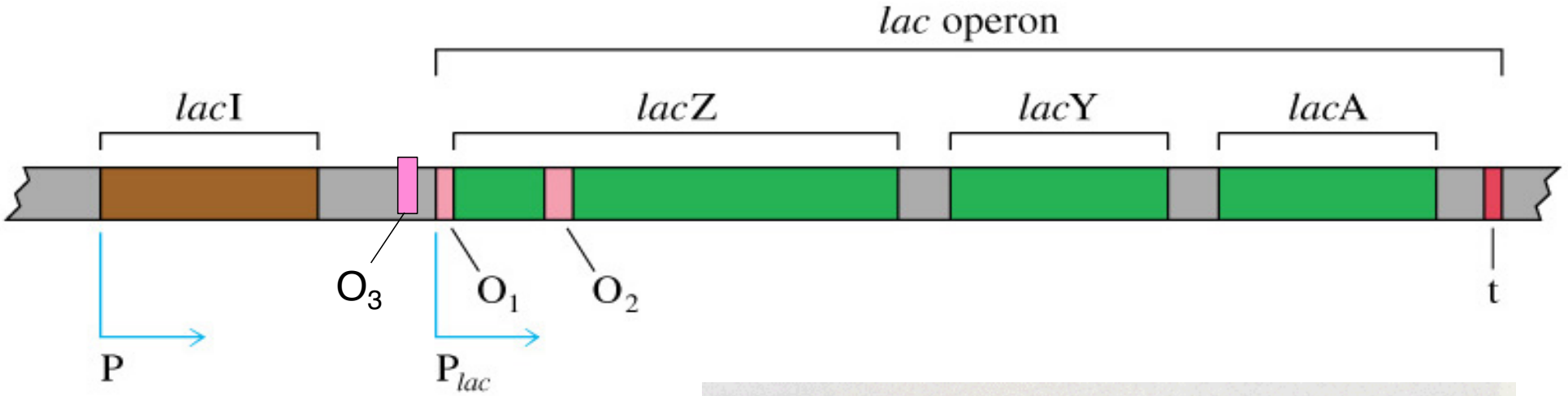
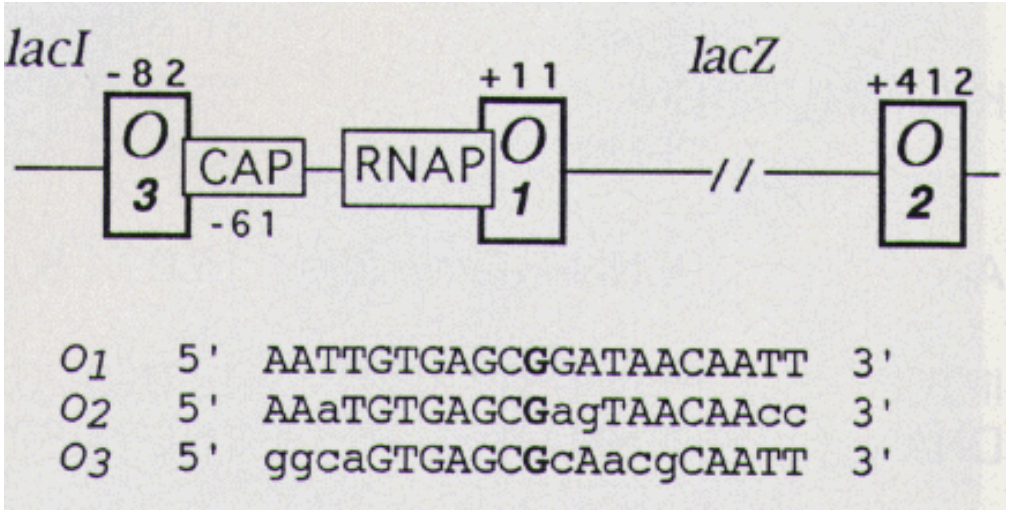
Lac repressor binding to DNA
-
linking structure and thermodynamics

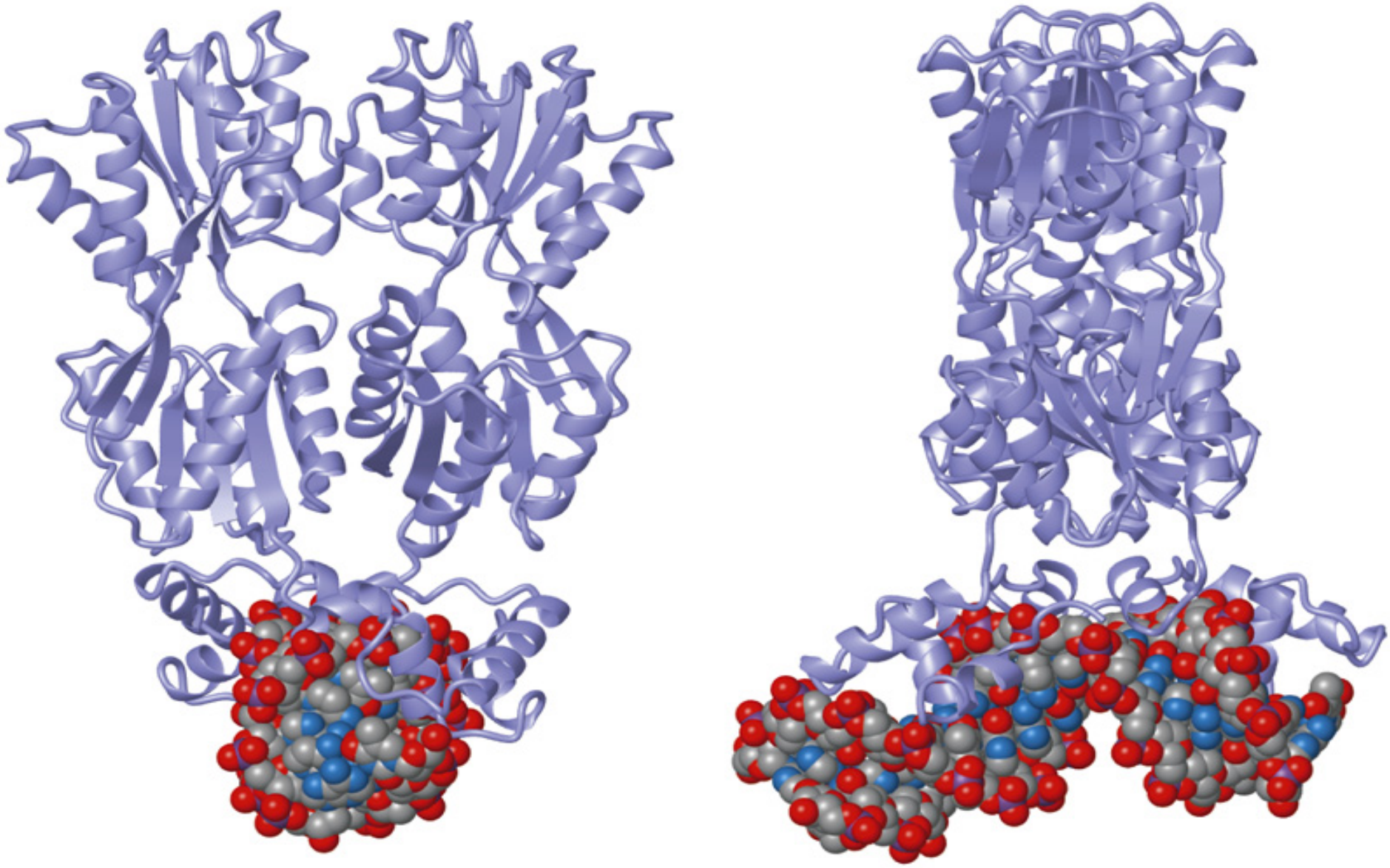
Organization of the genes regulated by Lac repressor, a transcription repressor protein in the bacterium E. coli



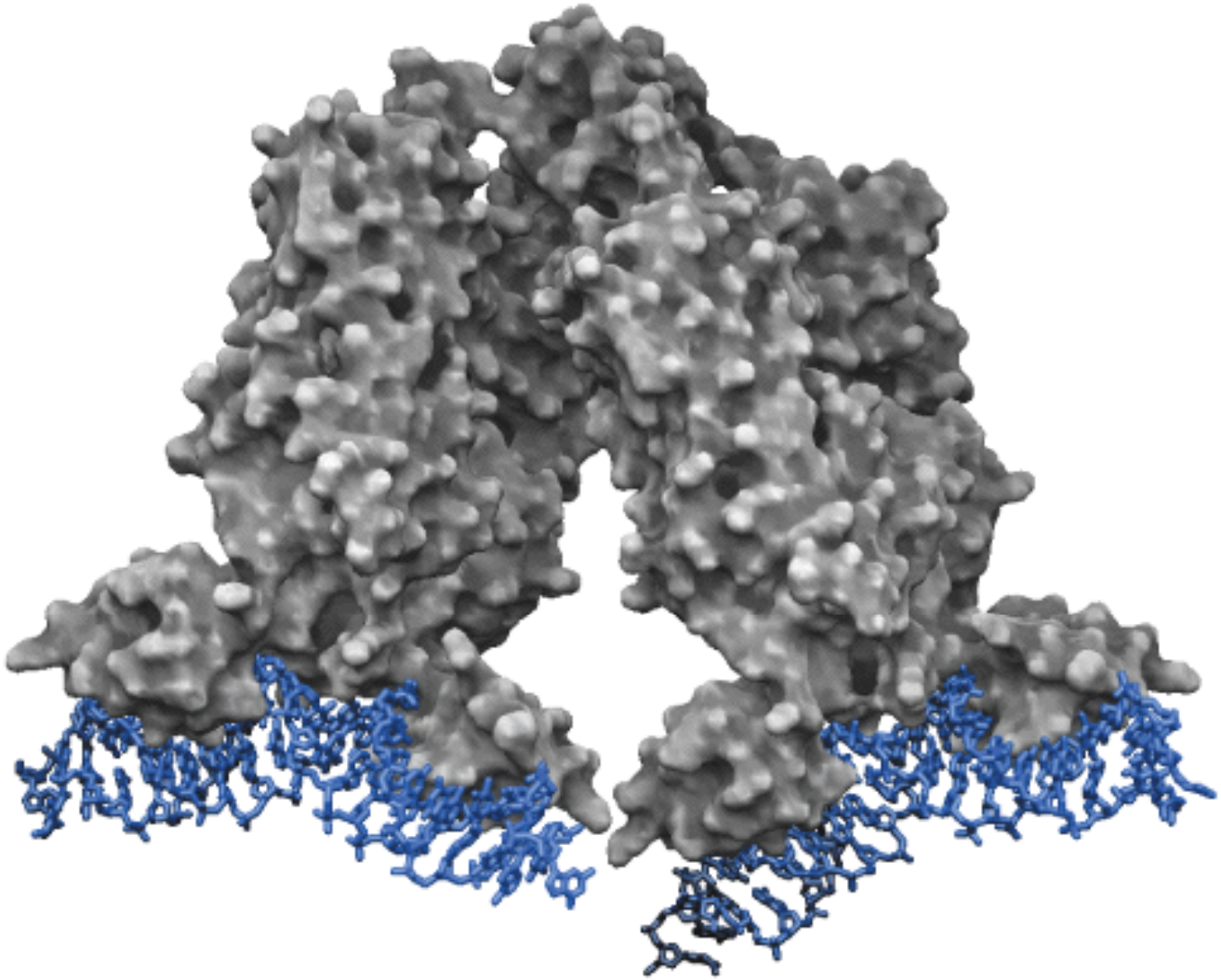
Lac repressor binds to the operators O_1, O_2 and O_3



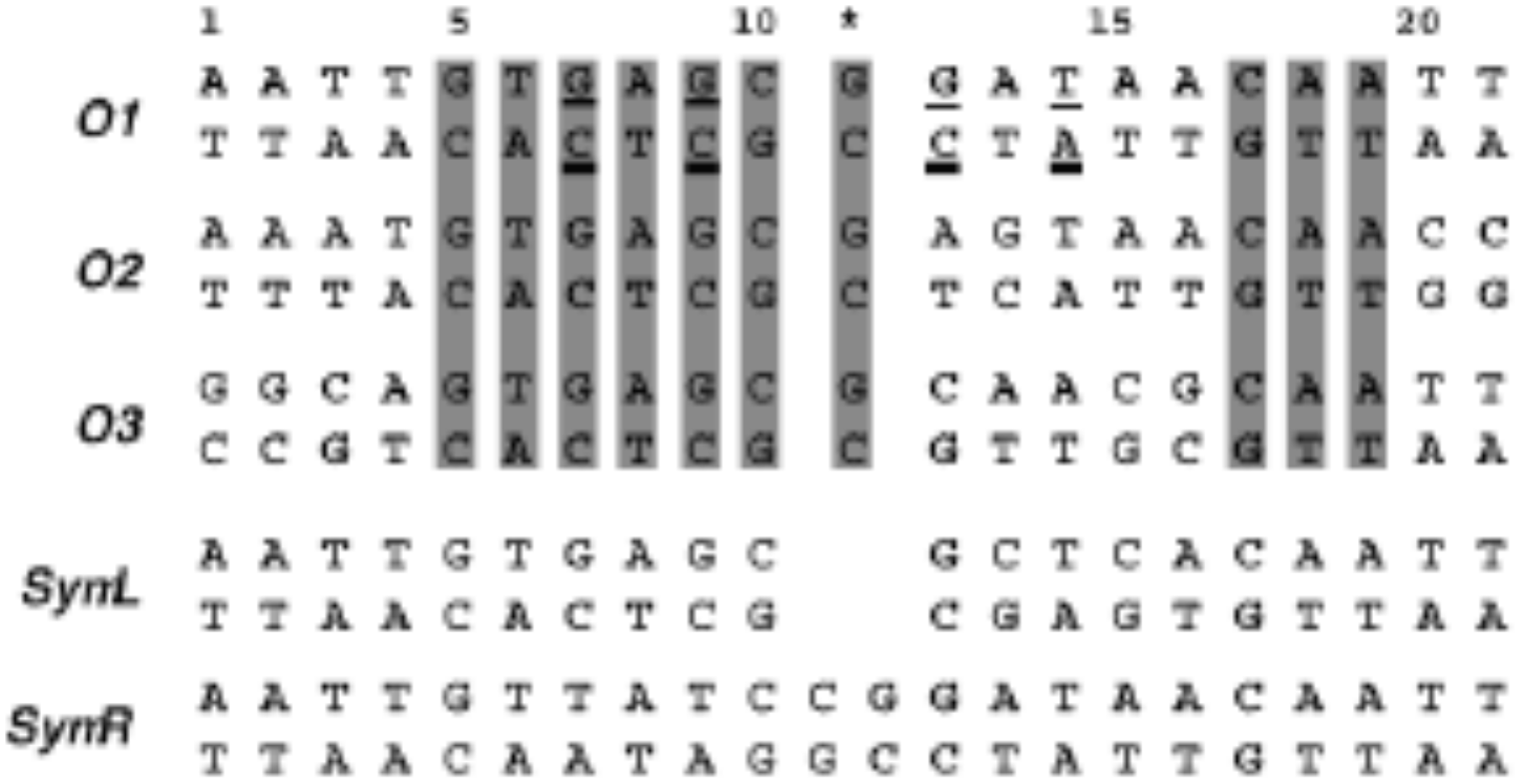
Molecular structure of E. coli lac repressor dimer



Molecular structure of E. coli lac repressor tetramer



Lac repressor binding sites



Lac repressor head piece (1-62) bound to SynL sequence

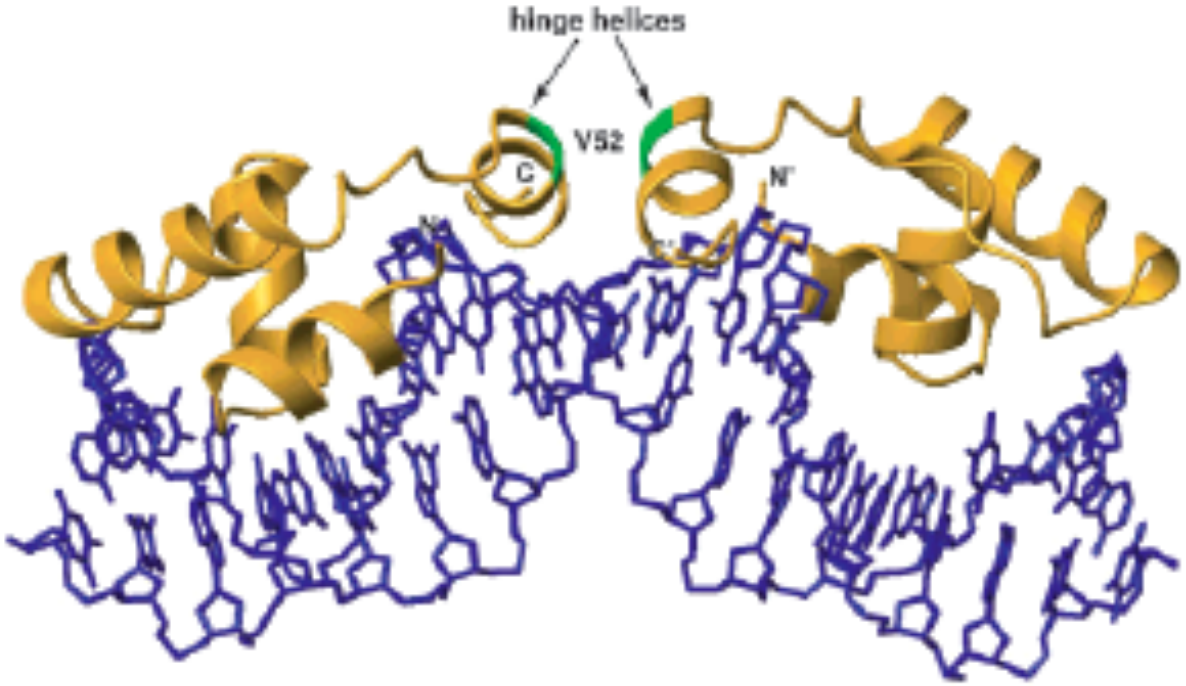


Figure 3. NMR solution structure of wild-type *lac* HP62 bound to the SymL operator (PDB access code 1CJG).[22] The two hinge helices (residues 50–58) bind to the minor groove of the SymL operator and bend significantly the DNA. On the basis of this structure, Val52 (green) was later replaced by a cysteine residue, so that a disulfide bond could link the two subunits, to yield a covalently linked dimeric lac that bound the natural operator *O1* with very high affinity.[35]

Lac repressor head piece (1-62) bound to the natural operator O1

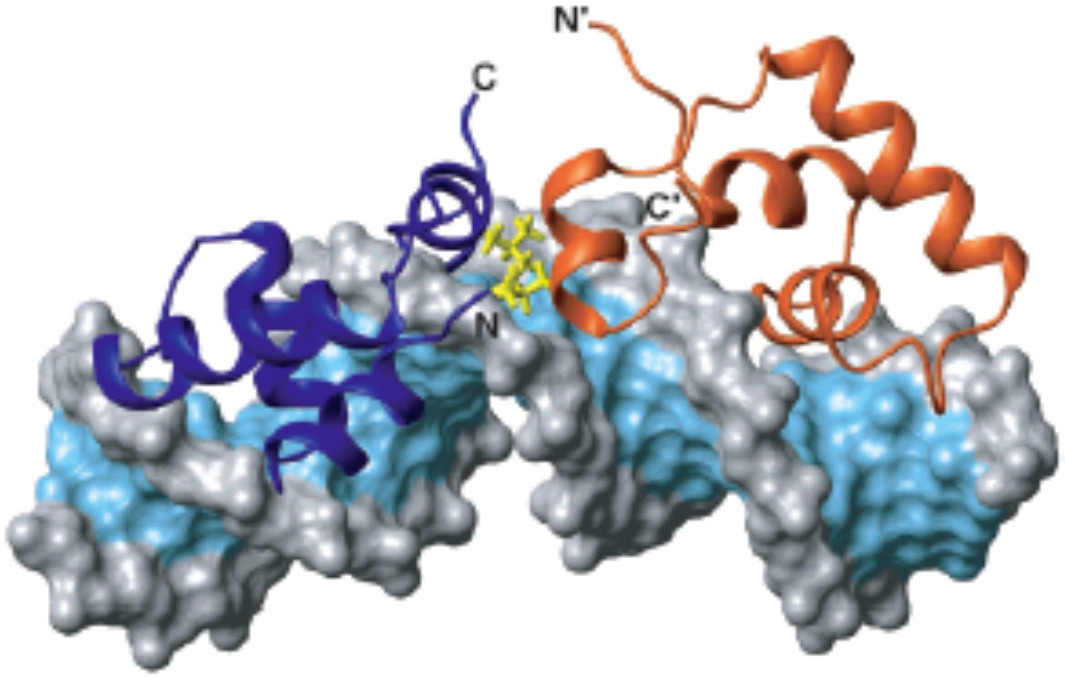
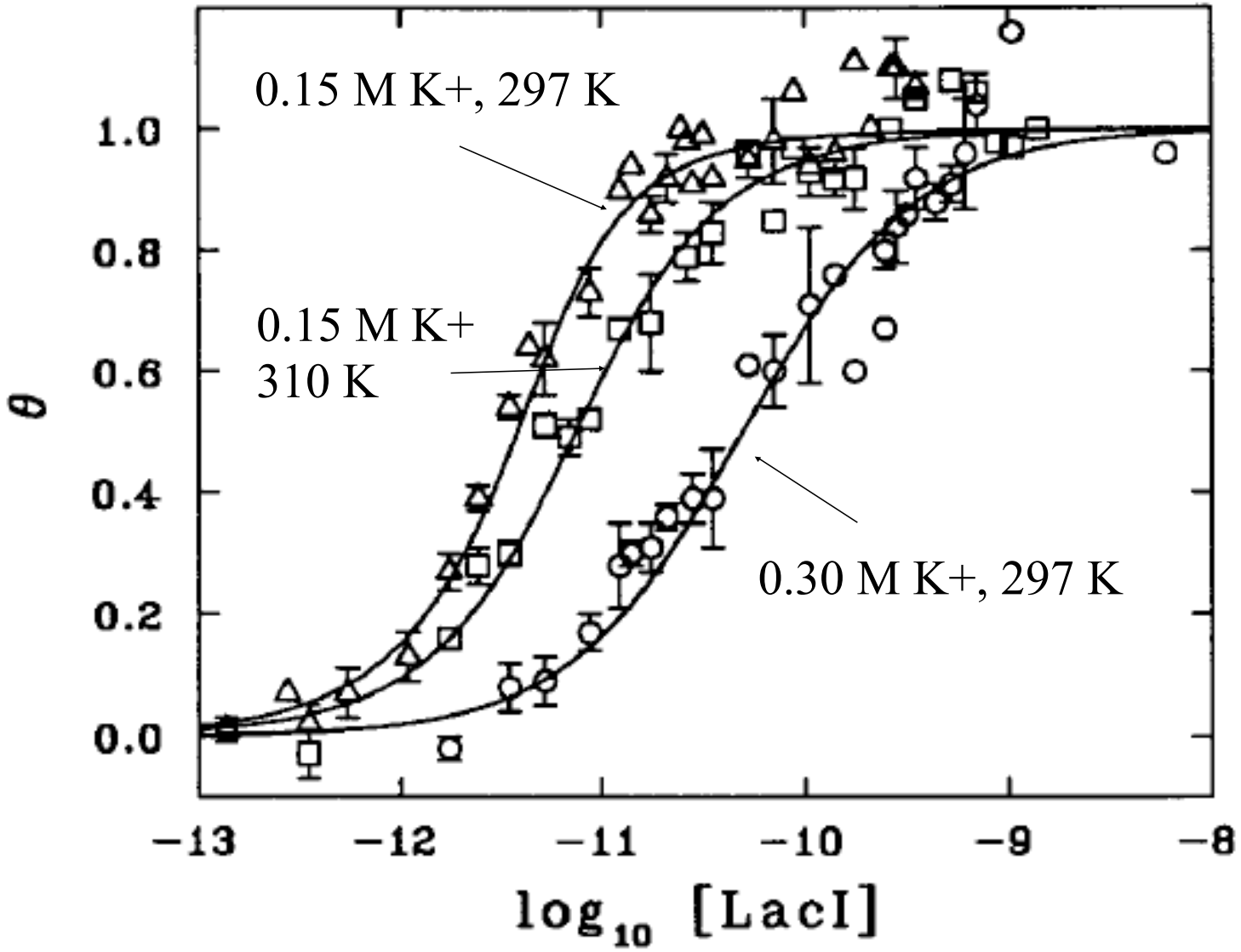


Figure 5. Three-dimensional structure of the dimeric *lac* DBD complexed to its natural *O1* operator (PDB accession code 1L1M).[41] A ribbon diagram of the protein is shown bound to the solvent-accessible surface of the operator. The left and right *lac* headpiece subunits are dark blue and dark orange, respectively. The major and minor grooves of the operator are light blue, and the ribose-phosphate backbone is gray. The side chains of Leu56 (shown in yellow) of both monomers protrude into the minor groove of the *O1* operator and introduce a ~36° kink centered between base pairs 10 and 11.

Binding titrations of symmetrical operator site with Lac repressor measured by filter binding assay



The influence of binding one ligand (ion) on binding of another ligand (protein) to the DNA is referred to as binding "linkage"

Wyman Linkage Relationship

J. Wyman (1964) Adv. Protein Chem. 19, 223

$$\frac{\partial \log K}{\partial \log x} = \Delta \overline{X}$$

Electrostatic (Coulombic) Interactions

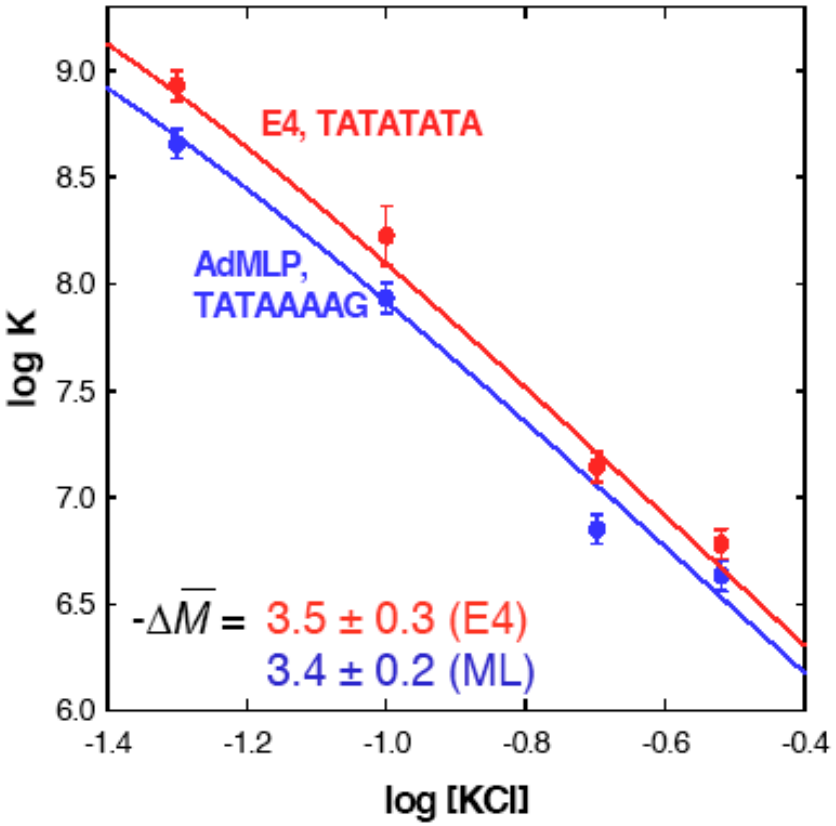
$$Interaction\ Energy = \frac{Z_a \cdot Z_b}{D \cdot r_{ab}}$$

Cation displacement

$$\frac{\partial \log K}{\partial \log [M^+]} = \Delta \overline{M}^+ = \varphi \overline{z}$$

fraction of charge neutralized = 0.88

number of salt bridges between DNA phosphates and positive amino acids



specific binding of TBP to different promoter sequences

Including the binding of ions in the reaction

Upon binding to the DNA *D* the protein *P* interacts with *n* phosphates so that $m = n \cdot \psi$ cations M^+ are displaced:

$$P + D \rightleftarrows PD + mM^+$$

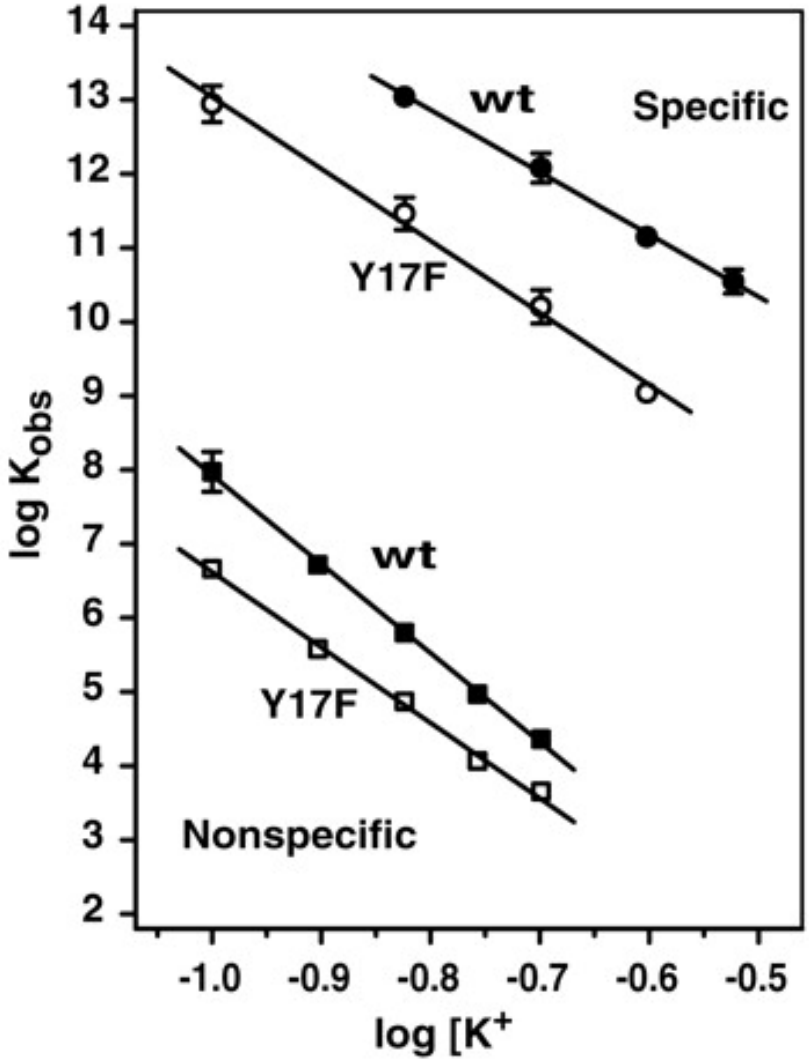
$$K = \frac{PD \cdot \left[M^+\right]^m}{P \cdot D}$$

However, we derive K from the concentrations of P, D and PD, thus

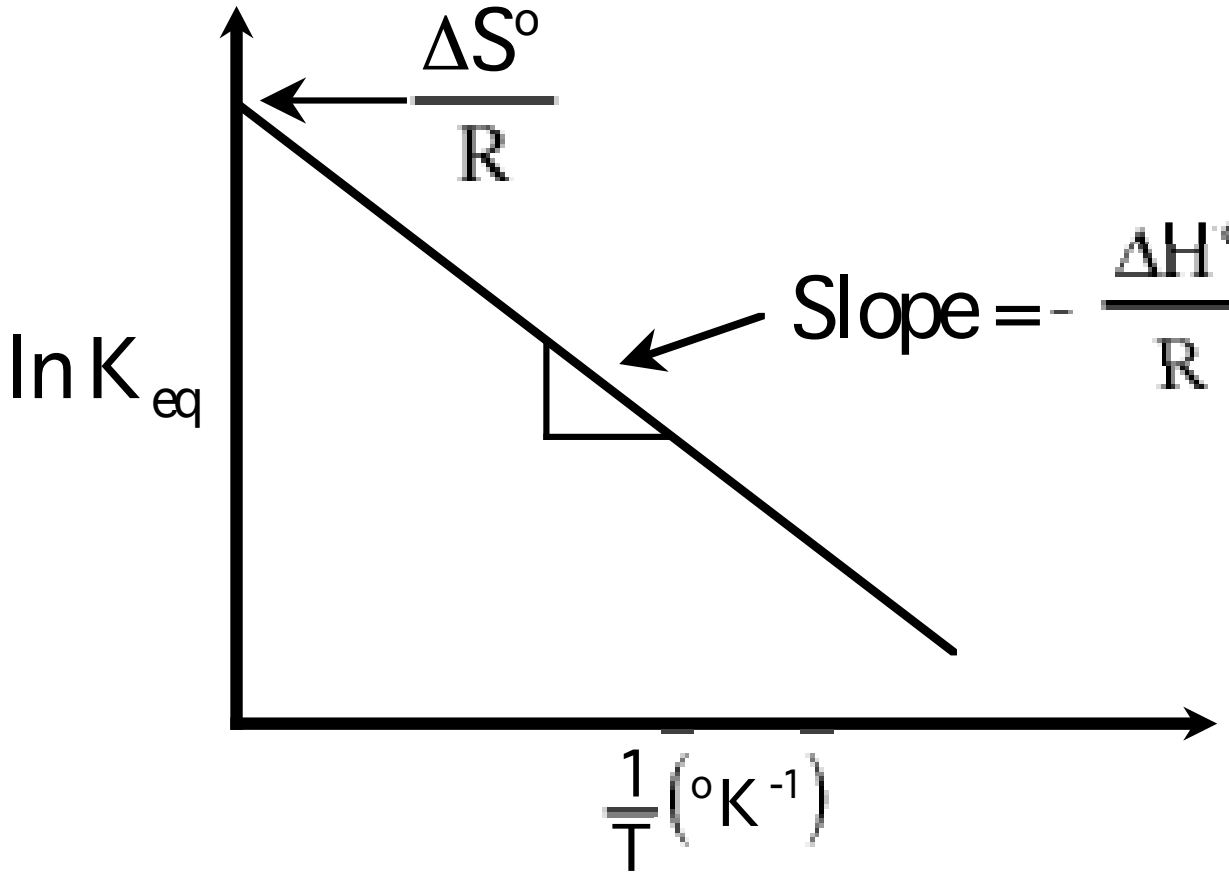
$$K_{obs} = \frac{\left[PD\right]}{\left[P\right] \cdot \left[D\right]} = \frac{K}{\left[M^+\right]^m}$$

$$\log K_{obs} = \log K - m \log \left[M^+\right]$$

Lac repressor binding involves 6-7 (specific) and 11 (nonspecific) charge-charge interactions



The temperature dependence of the binding constants reveals ΔH and ΔS in a van't Hoff plot if ΔH and ΔS are independent of temperature



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

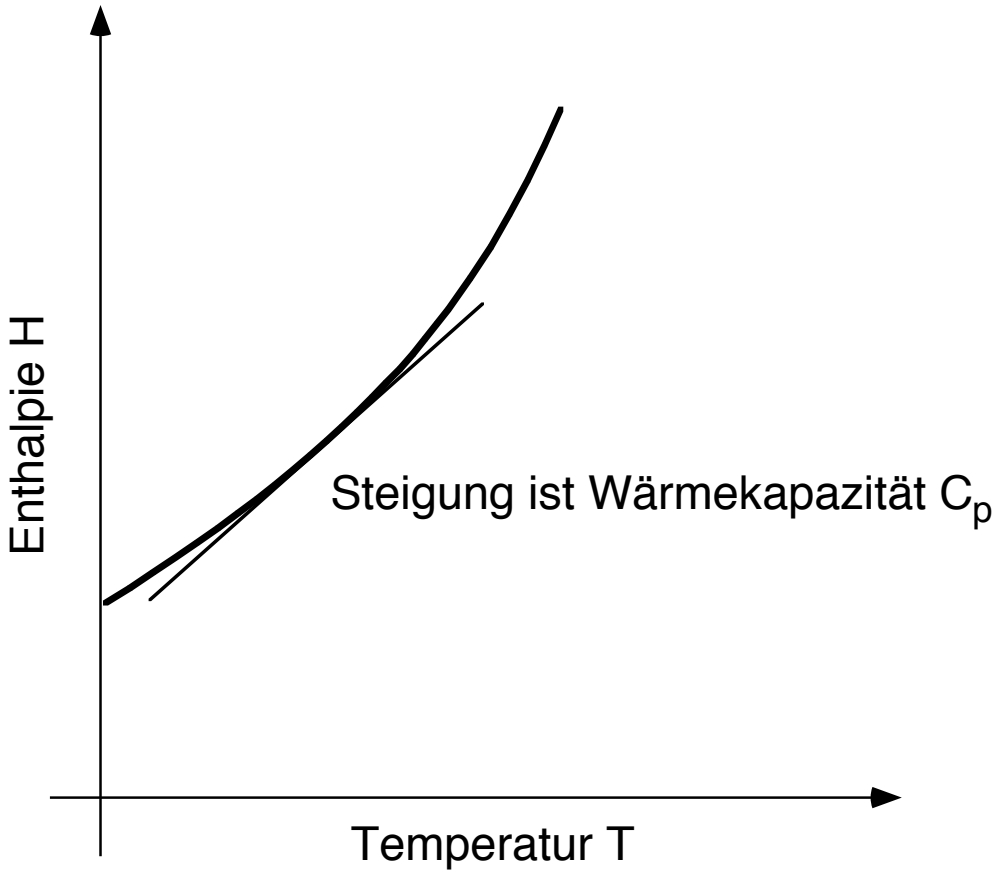
$$\Delta G = -R \cdot T \cdot \ln K_{eq}$$

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

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

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 and it can be determined from the derivative.

The heat capacity C_P describes the temperature dependence of ΔH and ΔS



$$C_P = \frac{\partial H}{\partial T} = \frac{T \partial S}{\partial T}$$

or if C_P is constant

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

which is a good approximation for the narrow interval from 0 to 40 °C usually studied

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

For two characteristic temperature T_H and T_S with

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

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

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

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

$\Leftrightarrow$

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

ΔC_p vs ΔA_{np} for protein folding

There is a linear correlation between the heat capacity change for protein unfolding and the buried non-polar surface area.

This relationship is identical to that seen for the transfer of hydrocarbons from aqueous solution to the pure liquid phase

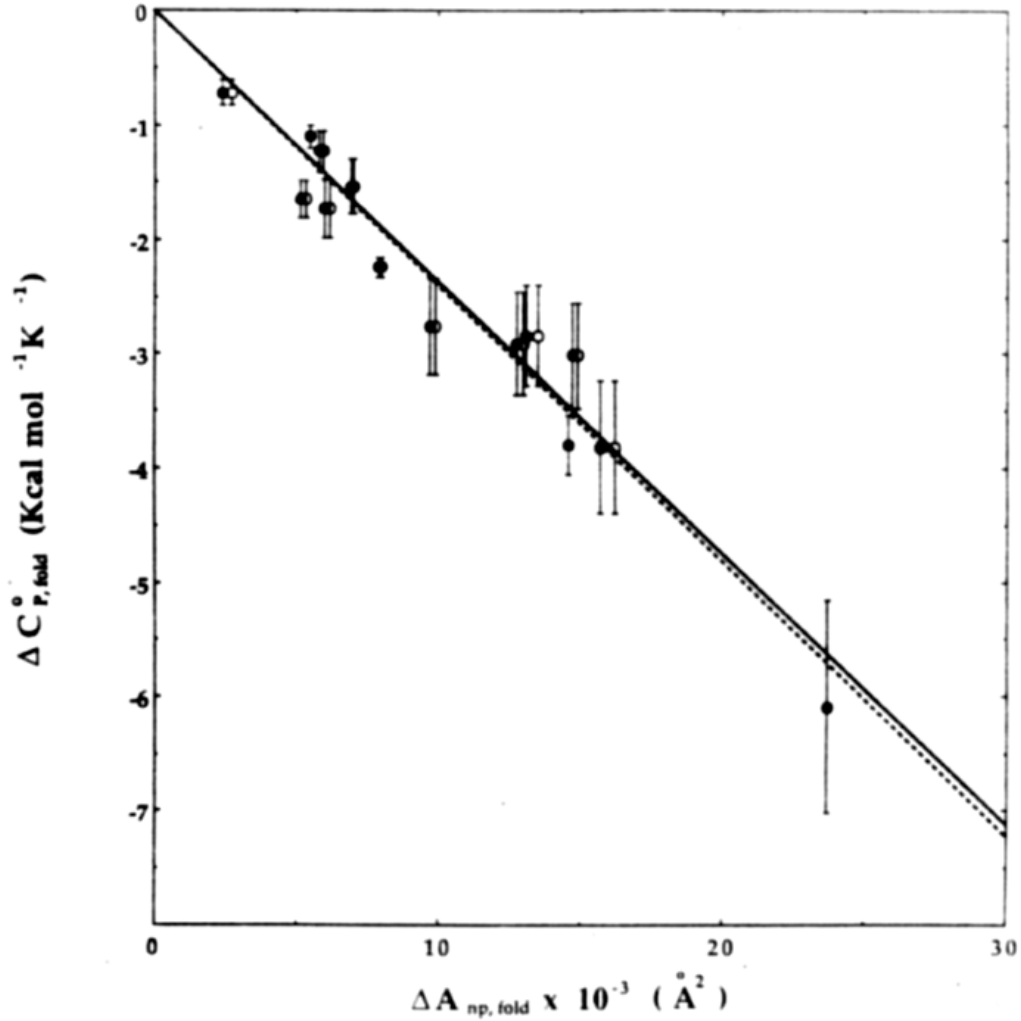
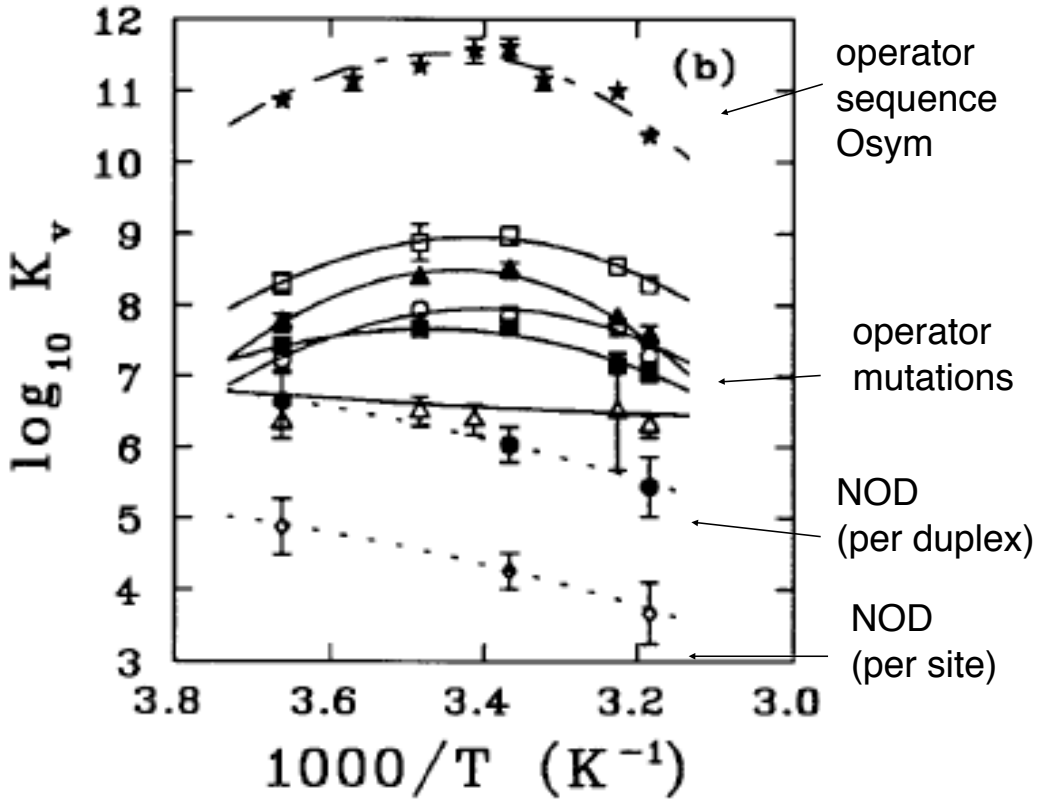


FIGURE 3: Standard heat capacity changes ($\Delta C^{\circ}_{p,fold}$) for the process of protein folding as a function of the reduction in water-accessible nonpolar surface area accompanying folding (ΔA_{np}). The denatured state is assumed to be in the extended β-form. The solid line is the weighted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

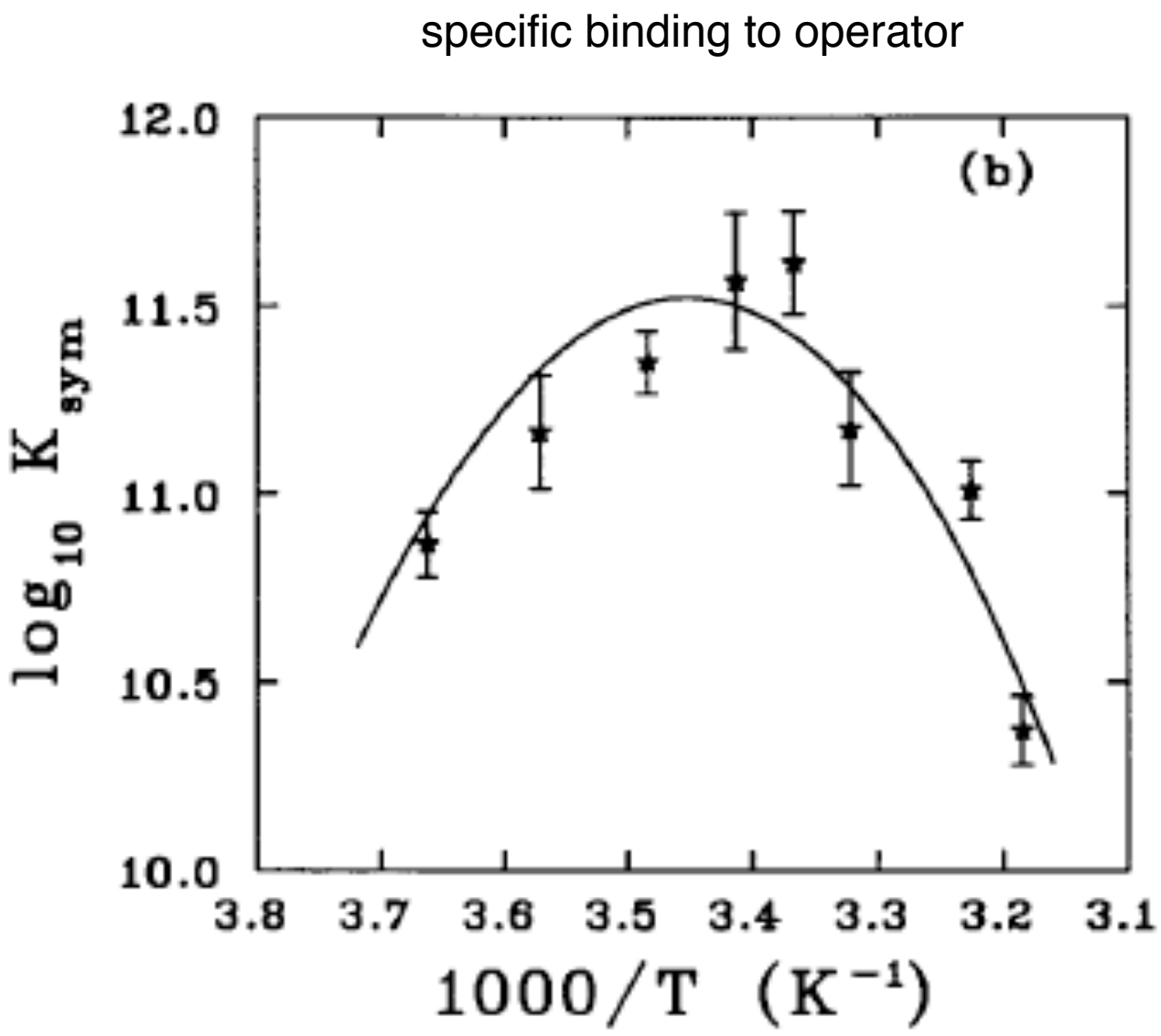
Variant Operators:

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

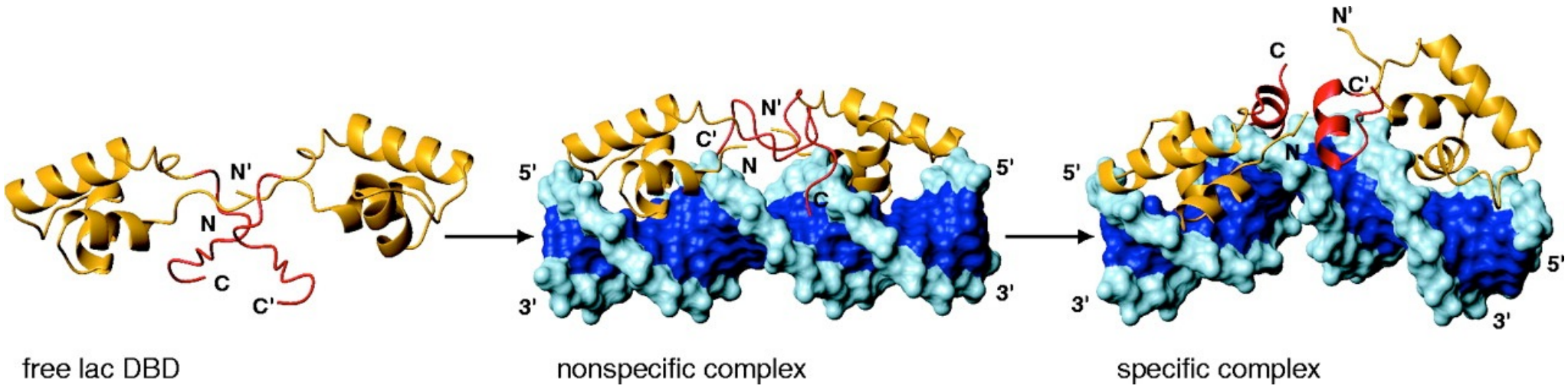
Nonoperator Fragment:

NOD	TCTAAGAGTTACTCTATCCG

Temperature dependence of Kd for specific binding of LacI repressor => induced folding



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

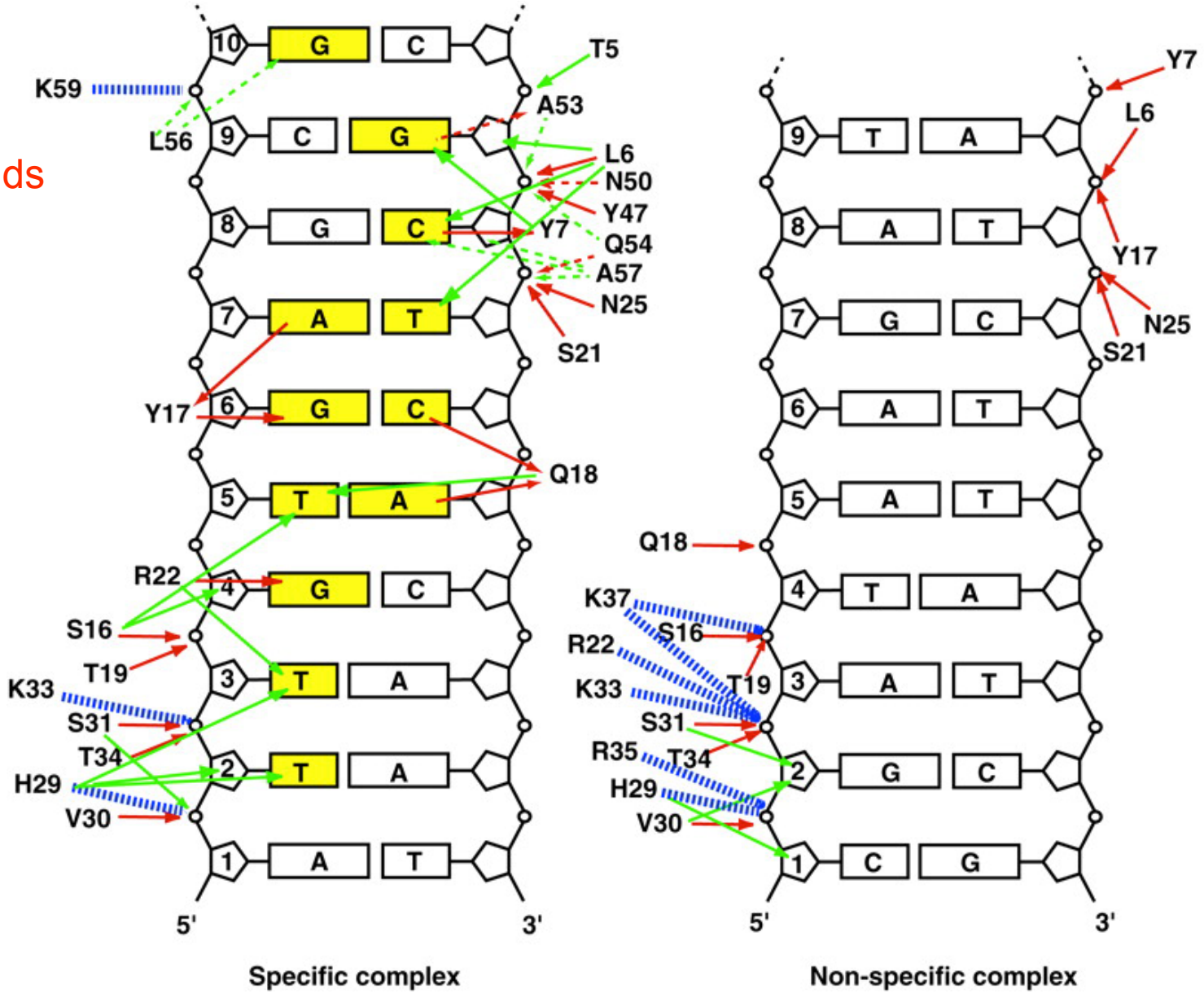


Specific (left) and nonspecific (right) protein-DNA contacts of Lac-DBD repressor with DNA

red:
hydrogen bonds

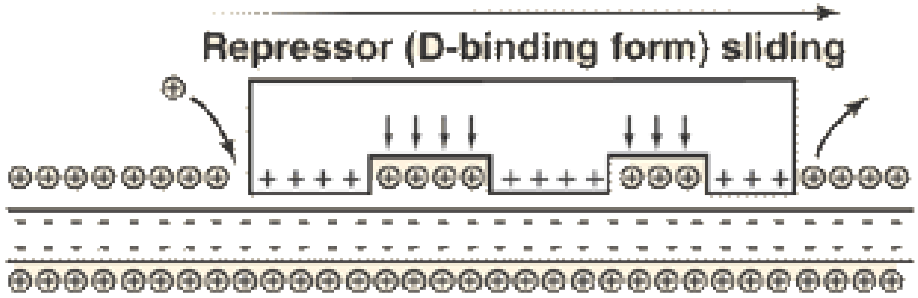
green:
hydrophobic
contacts

dashed blue:
electrostatic
contacts



Schematic models of the specific (RO) and nonspecific (RD) complexes of Lac repressor

RO and RD conformations are dynamic and interconvert with rate constants k_{RO} and k_{RD} transient curvature of DNA also in unspecific complex?



- Small arrows denote specific hydrogen bonding in the protein binding site. That are established in the specific complex upon folding of the hinge region
- Plus signs (+) denote basic side chains located in and around the same site. In the "down" position these groups are in "interactive contact" with the underlying dsDNA, and in the "up" position these contacts are broken.
- RO complex: 7 hydrogen bonds with the base pairs of the DNA operator site, only 6 electrostatic interactions with the charged DNA backbones.
- RD complex: 11 charge-charge interactions with the dsDNA backbone, but all the specific interactions with the DNA base pairs have been "withdrawn."
- curvature of DNA in the specific complex