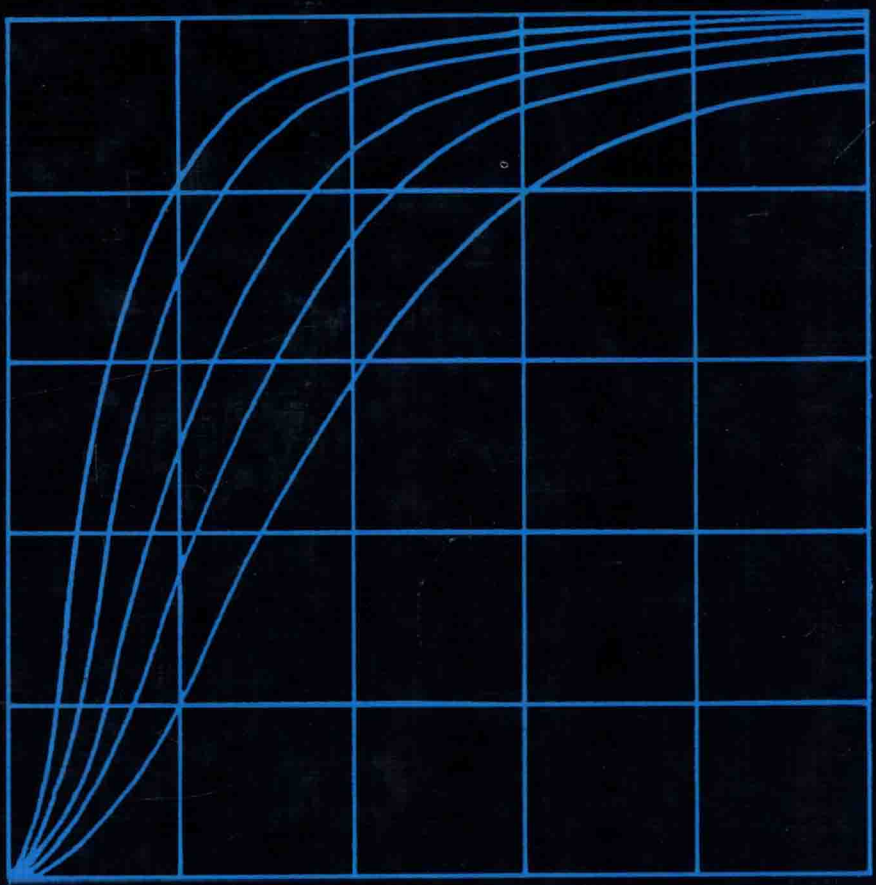
# PROTEIN INTERACTIONS

Gregorio Weber



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Chapman and Hall
New York London

First published in 1992 by
Chapman and Hall
an imprint of
Routledge, Chapman & Hall, Inc.
29 West 35 Street
New York, NY 10001

Published in Great Britain by

Chapman and Hall
2-6 Boundary Row
London SE1 8HN



Printed in the United States of America



**Library of Congress Cataloging in Publication Data**

Weber, Gregorio.
Protein interactions/Gregorio Weber.
p. cm.
Includes bibliographical references and index.
ISBN 0-412-03031-4
1. Protein binding. I. Title.
QP551.W38 1992 91-28667
574.19'245—dc20 CIP

**British Library Cataloging in Publication Data**

Weber, Gregorio
Protein interactions.
I. Title
547.75

ISBN 0412030314

# PROTEIN INTERACTIONS

Dedicated to Those Who Put Doubt Above Belief

# Preface

This book contains a discussion of concepts that facilitate the understanding of the simpler aspects of the interactions of proteins with physiological ligands and with other proteins.

I attempt here generalizations that often demand mathematical derivation and numerical computation, which I prefer to present graphically. In these days of naive confidence in computer calculations it seems necessary to restate that no computation can be more reliable than the concepts that underlie it, and that the most useful lesson that we have learned from computers is that the grammar is more important than the numbers.

At any time and in any scientific subject it is comparatively easy to master the concepts that govern what is already understood and widely practiced. It is far more difficult to appreciate that simple concepts, although demonstrably valid in known cases, cannot be extended to all systems regardless of complexity. Physical chemistry is an area of science greatly burdened by overconfidence in the universal value of simple rules, and in applying these to the proteins I have tried to make a clear distinction between what we can and cannot take for granted.

In my exposition I often stress the limits of our present knowledge of proteins on two counts. First, because awareness of present deficiencies is the origin of future knowledge. Second, because appreciation of our present limitations in the area of protein interactions provides a salutary antidote to the impression of perfection that the student of biochemistry is likely to receive from the amount of structural detail of the proteins that X-ray crystallography, and more recently magnetic resonance, have made available.

I have lived through the period of rapid expansion of the experimental techniques of protein investigation and have thus witnessed the first steps of many subjects that are now presented to the student without reference to their beginnings. When possible I have tried to refer to the original research rather than to recent reviews of these subjects. For it is not difficult to notice that the first observations contain in their naive exposition of facts and concepts much more than many subsequent elaborations.

Urbana, July 1991

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# I

# Thermodynamic Fundamentals

## Energy and Entropy

In discussions of energetics the word system denotes an isolated portion of matter of arbitrary composition. The energy, or more precisely the internal energy $U$ of a system is that characteristic property that is increased by the absorption of heat, $Q$ (measured in calories) and decreased by the performance of external work $w$ (measured in ergs or Joules) [1]. The first law of thermodynamics states that the changes in internal energy $dU$ are related to the changes in heat, $dQ$ and work, $dw$ in the manner

$$dU = dQ - dw \tag{1}$$

Any series of events that take the system out of its initial condition and finally brings it back to its original state perform a cycle in which $dU = 0$. We shall be exclusively concerned with system transformations, cyclic or otherwise, carried out at constant pressure $p$, whether this be the atmospheric pressure or a different one. For such cases

$$dw = p\,dV \tag{2}$$

where $dV$ is the change in volume of the system. Clearly $dw$ represents the work incurred in the expansion or contraction of the system against the constant pressure. It follows that

$$dU = dQ - p\,dV \tag{3}$$

If the internal energy remains unchanged ($dU = 0$)

$$dQ = p\,dV \tag{4}$$

an equation that postulates the equivalence of heat and mechanical work. The numerical equivalence is 1 calorie = 4.155 J or $4.155 \times 10^7$ ergs. It is convenient to express the internal energy of the system by means of the enthalpy $H$, a quantity somewhat different from $U$. A system of volume $V$ at pressure $p$ has an enthalpy

$$H = U + pV \tag{5}$$

A change in enthalpy entails

$$dH = dU + p\,dV + V\,dp \tag{6}$$

and from **(3)**

$$dH = dQ + V\,dp \tag{7}$$

The last equation indicates that the change in enthalpy equals the change in heat content at constant pressure.

The second law of thermodynamics starts with the definition of the entropy: If a system changes from an initial to a final state its change in entropy $dS$ is given by

$$dS = S_{\text{final}} - S_{\text{initial}} = \sum dQ_i/T_i \tag{8}$$

The entropy change is therefore the sum, with appropriate sign, of the changes in heat content taking place at temperature $T$ divided by the temperature at which they take place. The number of terms in the sum of Eq. **(8)** must be large enough so that $T_i$ is a virtual constant during the absorption of an amount of heat $dQ_i$. It was first deduced by Clausius that while the state of a system does not imply a unique value for the heat content it does define a unique entropy. Heat can be converted into work when a quantity of heat is transferred from a higher temperature, $T_h$ to a lower one $T_l$. In a cycle of heat transfer operations that returns the system to the original conditions (Carnot cycle) the yield of work $q$ is given by

$$q = \frac{\text{work performed}}{\text{heat transferred}} \leq 1 - (T_l/T_h) \tag{9}$$

The performance depends therefore on the absolute temperatures of the source ($T_h$) and sink ($T_l$) of heat and is always conspicuously less than 1. The second law of thermodynamics generalizes the incomplete transformation of heat into work by stating that in a spontaneous process (isolated system) external work cannot be performed at the expense of a decrease in

the entropy of the system. Accordingly in Eq. **(8)**

$$dS(\text{isolated system}) > 0 \tag{10}$$

In all spontaneous processes occurring in an isolated system the entropy must necessarily increase.

**The Thermodynamic Potentials**

The quantities $U$ and $H$ are uniquely determined by the variables of state $p$, $T$, $V$, and $S$ the latter being determined by the previous three variables and the *specific composition of the system*. $U$ and $H$ are potential functions in the sense that the difference between the initial and final values determines the capacity of the system to perform work when it passes from one state to another. To these thermodynamic potentials a third, the most important one for the chemist, must be added [2]. This is the free energy, or more specifically the Gibbs free energy $G$, formally defined by the relation

$$G = H - TS \tag{11}$$

Small changes in $G$ are related to those in $H$, $T$, and $S$ by

$$dG = dH - T\,dS - S\,dT \tag{12}$$

which on introduction of Eqs. **(7)** and **(8)** becomes

$$dG = V\,dp - S\,dT \tag{13}$$

It is seen in Table 1 that the potentials are determined by the values of $V$, $p$, $T$, and $S$. $p$ and $T$ are external variables in the sense that they do not belong to the system itself and $V$ defines an extensive property that can be varied without changing the relative proportions of the components. Thus the properties of the thermodynamic potentials that derive from the nature of the components of the system and their relative amounts are contained in the unique value of the entropy. Equation **(12)** gives one of the most important properties of the free energy: At constant temperature and pressure $G$ cannot change value unless $S$ changes. In a spontaneous process $dS > 0$. Therefore if such spontaneous process occurs at constant temperature and pressure $dG < 0$: the free energy of the system necessarily decreases. Any external work performed by the system under this condition must be done at the expense of its free energy. The importance of the thermodynamic potential $G$ becomes evident when we

*Table 1.* Summary of Thermodynamic Potentials

| Symbol | Independent variables | Important relations | Name |
|---|---|---|---|
| $U$ | $V, S$ | $T = dU/dS$<br>$dU = T\,dS - p\,dV$<br>$p = -(dU/dV)_S$ | Energy |
| $H$ | $p, S$ | $T = dH/dS$<br>$dH = T\,dS + V\,dp$<br>$V = (dH/dp)_S$ | Enthalpy |
| $G$ | $p, T$ | $V = dG/dp$<br>$dG = -S\,dT + V\,dp$<br>$S = -(dG/dT)_p$ | Free energy |

consider the relations:

$$dV = \left(\frac{dG}{dp}\right)_T; \qquad dS = -\left(\frac{dG}{dT}\right)_p \tag{14}$$

which permit the determination of the changes in volume and entropy from the changes in the Gibbs free energy with pressure, at constant temperature and with temperature, at constant pressure, respectively. A further important property of the free energy is contained in the Gibbs–Helmholz relation:

$$d\left(\frac{dG}{T}\right)\Big/d\left(\frac{1}{T}\right) = dH \tag{15}$$

This relation permits one to determine the enthalpy change of the system from the thermal coefficient of the free energy change. It is derived by noting that from **(11)** and **(14)**,

$$G = H + T\left(\frac{dG}{dT}\right); \qquad \frac{dG}{dT} = \frac{-dG/d(1/T)}{T^2}$$

which yield

$$\frac{G + [dG/d(1/T)]}{T} = \frac{d(G/T)}{1/T} = H \tag{16}$$

Replacing $G$ and $H$ by their finite increments $dG$ and $dH$ gives Eq. **(15)**. Relations **(14)** and **(15)** demonstrate that the changes in volume, entropy, and enthalpy occurring with a specified change of the system, for example, when a chemical reaction takes place, can be found through the changes in free energy with temperature and pressure. In the relation $G = H - TS$ only two of the three quantities $G$, $H$, and $S$ are independent. These may be taken to be $H$ and $S$, the enthalpy and entropy, respectively. Their relative importance, particularly as regards chemical reactions, may be conveyed by paraphrasing the words of Emden [3]: "in the factory of nature entropy has the position of manager, for it dictates the manner and method of the whole business, while energy merely does the book-keeping balancing credits and debts."

## Gibbs Free Energy and the Chemical Potential

In a homogeneous system made up of a single component a change in Gibbs free energy cannot take place, according to Eq. **(13)**, unless $dp <> 0$ or $dT <> 0$. Such a homogeneous system could well be called a "physical system." When several distinct components are present it is necessary to take into account the possibility of chemical reactions that can change their amounts, and therefore the value of $S$, although the system is isolated and remains at constant temperature and pressure. Let such "chemical system" involve components A, B, C, D capable of undergoing chemical reaction. Then, if in Eq. **(13)** $dT = dp = 0$, we have instead of $dG = 0$

$$dG = \mu(\mathrm{A})\, dn(\mathrm{A}) + \mu(\mathrm{B}) dn(\mathrm{B}) + \mu(\mathrm{C})\, dn(\mathrm{C}) + \mu(\mathrm{D})\, dn(\mathrm{D}) \quad \textbf{(17)}$$

In this equation $dn(\mathrm{A}) \ldots dn(\mathrm{D})$ are the molar amounts by which the components change in the chemical reaction. $\mu(\mathrm{A}) \ldots \mu(\mathrm{D})$ represent the changes in Gibbs free energy that take place in a change of 1 mol of the substance, under the conditions of composition, temperature, and pressure peculiar to the system. Equation **(17)** may then be considered as providing a definition of $\mu(\mathrm{A}) \ldots \mu(\mathrm{D})$, the "chemical potentials." At constant pressure and temperature the whole of the change in the free energy derives from the changes in the chemical potentials.

The biochemist—always—and the chemist—more often than not—are concerned with reactions taking place in solution. It is empirically known that chemical reactions in solution proceed to an extent determined by the concentrations of the reagents, a relation expressed in the so-called law of mass action [4]. Therefore the changes in the chemical potentials of the

reagents must depend on their concentration. This dependence is taken into account by writing the chemical potential in the form

$$\mu = \mu_0 + w(c, c_0) \tag{18}$$

According to (**18**) the chemical potential results from two contributions: $\mu_0$, the standard chemical potential, designates the part of the potential that can be known only through the chemical reactions in which the substance takes part when present at an arbitrarily fixed concentration $c_0$. This is commonly taken to be 1 mol/liter. The concentration dependent function $w(c, c_0)$ corresponds to the free energy change required to bring this 1 mol of reagent from the standards concentration $c_0$ to its actual concentration $c$. It is calculated from the dependence of the osmotic pressure on the concentration [5]. If the dissolved molecules behaved in an ideal fashion they would exert on a semipermeable membrane separating them from pure solvent an osmotic pressure $\pi$ dependent on the concentration in the same manner as the pressure exerted on the walls of the container by the molecules of an ideal gas. The pressure developed at a molar concentration $c$ would then equal

$$\pi = cRT \tag{19}$$

where $R$ is the familiar gas constant. The free energy change in the dilution from $c$ to $c_0$ equals the osmotic work

$$w = \int_c^{c_0} \pi \, dV \tag{20}$$

A solution of concentration $c$ supposes a molar volume $V = 1/c$. Therefore introducing the relation $dV = -dc/c^2$ and Eq. (**19**), Eq. (**20**) becomes

$$w = \int_c^{c_0} \frac{-RT\,dc}{c} = RT \ln\left(\frac{c}{c_0}\right) \tag{21}$$

and for $c_0 = 1$

$$\mu = \mu_0 + RT \ln c \tag{22}$$

Real solutions do not follow this simple equation except at low concentrations. In the case of macromolecules at least, a plot of $\pi/c$ against $c$ has typically the form shown in Figure 1 and the dependence of $\pi$ on $c$ is

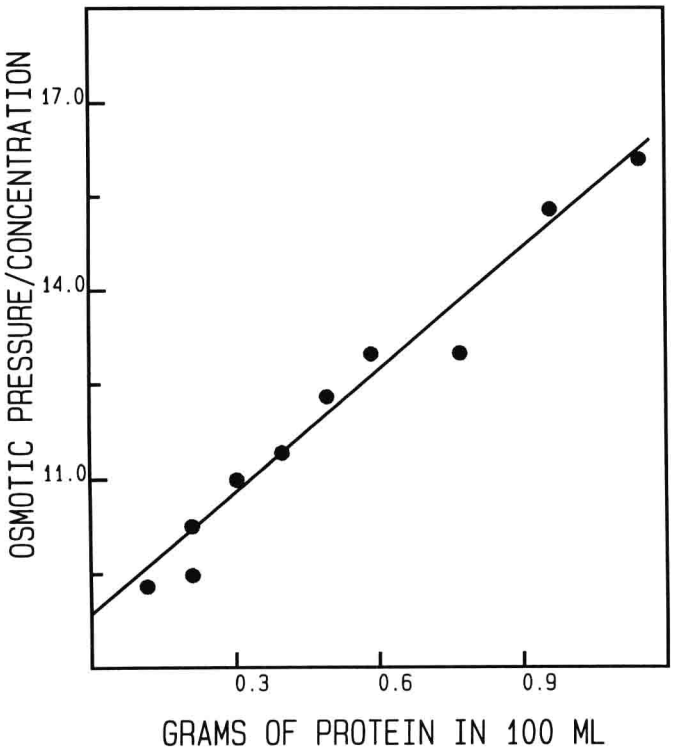


*Figure 1.* Plot of $\pi/c$ against $c$ for glycinin, a soya bean protein, in 4 $M$ guanidine hydrochloride showing the importance of the second virial coefficient [$B_1$ in Eq. (**24**)]. From numerical data of ref. [5], p. 93.

given by a virial expression:

$$\frac{\pi}{c} = RT\left(1 + B_1 c + B_2 c^2 + \cdots\right) \tag{23}$$

where the virial coefficients $B_1, B_2, \ldots$ are specific to each system. The integral of Eq. (**21**) takes the form

$$\frac{w}{RT} = \ln[c/c_0] + B_1(c - c_0) + B_2\left(c^2 - c_0^{\,2}\right)/2 + \cdots \tag{24}$$

The activity coefficient $\gamma$ may be defined by the relation

$$\ln \gamma = B_1(c - c_0) + B_2(c^2 - c_0^2) + \cdots \tag{25}$$

which gives

$$w = RT \ln(\gamma c); \qquad \mu = \mu_0 + RT \ln(\gamma c) \tag{26}$$

The activity coefficient $\gamma$ tends to unity in the region in which $B_1(c - c_0) \ll 1$. It may differ from 1 at concentrations as low as 0.01 m$M$ for polyelectrolyte macromolecules and 0.01 $M$ for ordinary electrolytes [6]. Uncharged molecules in good solvents may exhibit $\gamma = 1$ at concentrations of 0.1 or even 1 $M$. Since very few solutions have $\gamma = 1$ at a molar concentration, an activity coefficient of 1 is arbitrarily assigned to the standard concentration $c_0$ appearing in equations like (**21**). In the binding of ligands to 0.1 m$M$ protein in solutions which are 0.05 $M$ or more in electrolyte concentration it is customary, and overall correct, to assume $\gamma = 1$. As these conditions are reached in most of the experimental circumstances of interest we shall assume that the general situation is one in which the simpler form of the chemical potential, $\mu = \mu_0 + RT \ln(c)$ may be used.

We note three important properties of chemical potentials:

1. They are extensive quantities that refer, by convention, to 1 mol. A fraction $dn$ of 1 mol has a chemical potential $\mu\, dn$.
2. Of the two terms forming the chemical potential the standard term represents the "true" chemical potential, in the sense that it is this part that determines the chemical behavior of the substance, and can only be known through it. The concentration-dependent term is ideally a universal function of the concentration expressing the osmotic work required to bring the standard to the actual concentration.
3. The standard chemical potentials are not absolute constants. Not only do they depend on temperature and pressure but in principle they depend on *all the other components of the system*. This last circumstance is of limited importance for reactions between very simple molecules to the extent that it receives little comment in texts of chemical thermodynamics. It is customary to define a "standard state" from which, by the simple operations of the potential functions, one may derive the correct chemical potential in media of different composition. Though legitimate in principle this view does not lend itself to practical application in the case of molecules as complex as the proteins. We have to assume that for

them the values of the standard chemical potentials are defined only for media of precise composition and more often than not we are not able to accurately measure, or even estimate, the difference between these values in media of quite close chemical composition (see Chapter II).

**Change in Gibbs Free Energy in a Chemical Reaction**

The relation and significance of the two parts that make up the chemical potential become clear in describing the free energy change that takes place in a chemical reaction. Let this reaction involve the conversion of $n$ mols of reactants (A and B) into products (C and D); $n$ represents the molar amount transformed, the same for all components. If the initial amounts of the components are $n_A \ldots n_D$, after reaction has taken place A and B have decreased by an amount $n$ and C and D have increased by the same amount:

$$G = \mu(A)(n_A - n) + \mu(B)(n_B - n) + \mu(C)(n_C + n) + \mu(D)(n_D + n) \tag{27}$$

From the last equation it follows that

$$\frac{dG}{dn} = \mu_C + \mu_D - \mu_A - \mu_B \tag{28}$$

or

$$\frac{dG}{dn} = \mu_0(C) + \mu_0(D) - \mu_0(A) - \mu_0(A) - \mu_0(B) + RT[\ln(\langle C\rangle\langle D\rangle)/(\langle A\rangle\langle B\rangle)] \tag{29}$$

$\langle A\rangle \ldots \langle D\rangle$ are the *arbitrary* molar concentrations obtaining *while n mol react*. Therefore, the differential coefficient $dG/dn$ on the left-hand side of **(28)** stands for the finite free energy change $dG$ in the conversion of 1 mol of A and B into 1 mol of C and D *under the condition that the concentrations* $\langle A\rangle \ldots \langle D\rangle$ *remain the same during the conversion*. We shall in the following denote the finite changes in $V$, $S$, and $G$ that take place when 1 mol of the components of a system passes from a well-defined initial state to an equally well-defined final state by the symbols $\Delta V$, $\Delta S$, and $\Delta G$, respectively. The term $\mu_0(C) + \mu_0(D) - \mu_0(A) - \mu_0(B)$ in **(29)** corresponds to the change in Gibbs free energy of the system when molar amounts of A and B are substituted by molar amounts of C and D. It is