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Review Article

An Outlook on Biothermodynamics. II. Adsorption of Proteins

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Abstract

The application of concepts, principles, and methods of thermodynamics of equilibria and processes to bioengineering systems has led to a new and growing field: engineering biothermodynamics. This article is the second in a series devoted to presenting to the biophysical chemist and the biochemical engineer the fundamentals and also the possibilities of this field, the latter aspect being demonstrated by examples. Here we will elaborate on the thermodynamics of adsorption phenomena of proteins on solid surfaces. Equilibria and processes of single- and multi-component protein solutions including an inert sorbent surface to adsorb proteins will be considered. Also, phenomena such as multi-contact adsorption of a (big) protein on a surface and the (often irreversible) denaturation process of a protein after adsorption will be discussed in brief.

1. Introduction

Proteins can adsorb on or stick to nearly every solid or liquid surface. This phenomenon is due to the (normally) heterogeneous structure of the surface of a protein, which includes hydrophilic as well as hydrophobic atomic groups [1]. Interestingly, proteins normally do not adsorb on each other, but when they do, they may form string- or sphere-like aggregates, thus losing their biochemical or physiological properties, which may in turn lead to disease. We will discuss this phenomenon in a subsequent paper of this series.

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Adsorption phenomena of proteins are of importance in many fields of biotechnology and related natural sciences. Examples are:

– immobilization of proteins, enzymes, and other biomolecules on surfaces to keep them or separate them from other components in a broth;

– use of adsorbed proteins in diagnostics as biosensors;

– biofouling and/or contamination of surfaces by adsorbed proteins as for example casein (milk protein) at the bottom of a pot of milk during heating;

– storage of proteins in pores of a carrier material for controlled release (drug targeting);

– separation of proteins in downstreaming processes (chromatography);

– and many more [2–4].

An interesting example with considerable technical potential is provided by the common sea barnacle (order Cirripedia). This salt-water preferring small shell is known to produce a certain protein that serves as a glue allowing the creature to attach itself to surfaces of all kinds, especially ships' bottoms. A special feature of this protein-glue is its resistance against water, whereas most glues used today are subject to water corrosion, leading finally to a breakdown of the adhesive properties of the glue.

In the following, we will restrict discussion to adsorption phenomena on inert solid surfaces; that is, liquid–gas surfaces will not be considered. We start by outlining the thermodynamics of the adsorption phenomena. Next, we will discuss the Langmuir adsorption isotherm and the related kinetics, as these are of practical importance for protein adsorption phenomena. Single protein component systems are presented first, followed by a multi-component systems analysis. As proteins are very large molecules compared to molecules of most solvents used today, it may happen that a protein in an adsorbed state on a solid surface has not only one but several contact regions, i.e., atomic groups on its surface being in contact with such groups from the surface of the solid sorbent. This is the phenomenon of multi-contact adsorption, which leads to considerable changes in both the equilibria states and the kinetics of adsorption, as desorption of the protein is more difficult than in a single-contact situation. In a first approach, we always assume the adsorption process to be reversible, i.e., we assume a protein desorbing from a surface to be in the same state as prior to adsorption. As this assumption often fails in practice, we will finally consider a combined adsorption and denaturation process of a protein and also provide a special example for this.

2. Adsorption thermodynamics

As proteins often are dissolved in an aqueous solution, we first will discuss some basic thermodynamic concepts and equations of adsorption phenomena from liquid solutions. Then we will present several adsorption isotherms, i.e., relations describing the amount of a protein adsorbed on a given surface or amount of material – a so-called sorbent – at a given temperature and concentration of the protein in the surrounding liquid phase. After this, we will also present simple models to describe the kinetics of the adsorption or desorption process. We always start with single protein component phenomena and then extend the formalism to multi-component protein systems, as they are more prevalent in practice.

The basic experiment to prove adsorption of a protein from a solution or "formulation" on a solid surface is sketched in Figure 1: A certain amount of inert but possibly porous material of mass (m^s) is put into the – say – aqueous solution consisting of (n_0^f) moles of protein, (n_w) moles of water and having the volume (V^f) at temperature (T) and pressure (p). After immersion of the sorbent, part of the protein will stick to its surface, thus lowering the protein concentration in the solution. Naturally, some water will either penetrate macropores of the sorbent or even be adsorbed on its meso- and micropores. However, we assume that this amount of water (n_w^a) is always much smaller than (n_w), i.e., ($n_w^a \ll n_w$). The validity of this assumption and necessity for possible corrections can be easily checked by immersing the sorbent in pure water only and weighing it after removing it from the water [5].

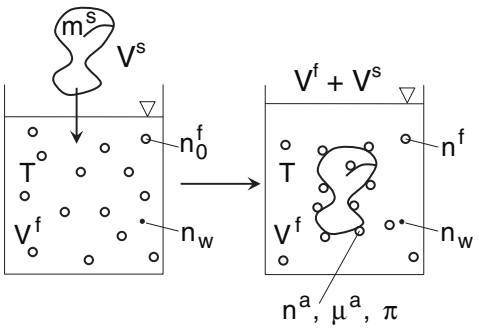

Figure 1 Inert solid material (m^s) being immersed in an aqueous solution (n_w) of protein (n_0^f) at temperature (T) and pressure (p). The amount of protein adsorbed on the inert surface of the sorbent is designated by (n^a); the amount of protein still in solution, by (n^f).

The molar balance of the protein is

$$n_0^f = n^f + n^a = \text{const.} \tag{1}$$

Here (n^f) is the amount of protein still dissolved in the water and (n^a) is the amount of protein adsorbed on the surface of the sorbent material. Introducing the protein's bulk concentration prior to and after immersion of the sorbent material by

$$c_0 = \frac{n_0^f}{V^f}, \quad c = \frac{n^f}{V^f}, \tag{2}$$

one can calculate (n^a) from Eq. (1) as

$$n^a = (c_0 - c)V^f \tag{3}$$

if the protein's concentrations ($c_0 \geq c$) have been measured and (V^f) is known. For this, a variety of experimental methods is available. We mention only spectroscopy here, i.e., UV fluorescence, light scattering, and light absorption methods. Also, dielectric permittivity or high-frequency rheological measurements, or calorimetric methods, i.e., measurements of heat capacities or protein-specific enthalpies can be used. Some proteins also immerse ions or atomic groups upon adsorption. This effect may also be used for (probably irreversible) adsorption measurements [6, 7].

Considering now the system in Figure 1 consisting of the protein in the aqueous solution with concentration c, the protein (n^a) being adsorbed on the surface of the sorbent material with sorption active area (A), constant mass ($m^s \simeq A$), and constant volume (V^s), we can formulate the condition for thermodynamic equilibrium in the usual way, namely by minimizing the free energy F of the whole system at constant temperature (T), total volume of the system ($V^f + V^s$), and sorption active area (A). Assuming F to be the sum of the free energy of the protein containing water phase (F^f) and the free energy of the protein adsorbate (F^a), we have

$$F^f + F^a \to \text{Min}, \tag{4}$$

$$T = \text{const}, \quad A = \text{const}, \quad V^f = \text{const}, \quad V^s = \text{const}. \tag{5}$$

Here F^f is the free energy of the protein solution with Gibbs equation

$$dF^f(T, V^f, n^f, n_w = \text{const}) = -S^f dT + p^f dV^f + \mu^f dn^f. \tag{6}$$

Similarly we have for the free energy (F^a) of the adsorbed protein

$$dF^a(T, A, n^a) = -S^a dT + \pi dA + \mu^a dn^a. \tag{7}$$

Here (π) is the so-called spreading pressure of the adsorbed protein phase to be calculated from its thermal equation of state (EOS),

$$\pi = \pi(T, A, n^a). \tag{8}$$

The minimization condition,

$$d(F^f + F^a) = 0, \tag{9}$$

delivers in view of Eqs. (1) and (5) the equilibrium condition

$$\mu^a(n^a, A \simeq m^s = \text{const}, T) = \mu^f(c = n^f/V^f, T), \tag{10}$$

from which on principle the amount of protein adsorbed, i.e., the so-called adsorption isotherm, can be calculated as

$$n^a = n^a(A \simeq m^s, c = n^f/V^f, T). \tag{11}$$

Note that the external pressure (p) of the system is implicitly included in this result as the volume of the fluid phase depends on it via the fluid's thermal EOS:

$$V^f = V^f(n_w = \text{const}, n^f, p, T). \tag{12}$$

Also, the pressure will influence the conformation of the protein and hence its tendency for adsorption. But this is not taken into account explicitly.

For practical use of the equilibrium condition (10), it is necessary to relate the chemical potential of the adsorbed phase (μ^a) with the thermal equation of state (EOS) of the adsorbate (8), where (π) is the so-called spreading pressure, i.e., a measure for the tendency of the adsorbed proteins to spread out on the available surface (A) of the sorbent material. It is related to the surface tension of the adsorbate but cannot be measured directly [8].

For this purpose, we consider the Gibbs–Duhem equation following from the total differential of the free energy of the adsorbed phase,

$$dF^a = d(\mu^a n^a - \pi A) = \mu^a dn^a + n^a d\mu^a - \pi dA - A d\pi, \tag{13}$$

and the Gibbs equation (7) as

$$A d\pi = n^a d\mu^a. \tag{14}$$

In view of Eq. (13) and the side conditions (5), this relation can be integrated to give a representation of the chemical potential of the adsorbed phase,

$$\mu^a(n^a, T, A = \text{const}) - \mu_0^a = \int_{n_0^a}^{n^a} \frac{1}{n^a} \left(\frac{\partial \pi}{\partial n^a} \right)_{A,T} dn^a. \tag{15}$$

Here (n_0^a, μ_0^a) are the mole number and the chemical potential of the adsorbate in a certain (isothermal) reference state.

3. The ideal protein adsorbate

For the simplest case of an "ideal adsorbate", we have the thermal EOS, which is similar to the ideal gas EOS, namely

$$\pi A(m^s, T) = n^a RT, \tag{16}$$

with the – possibly temperature-dependent – adsorption area $A(m^s, T)$ and the universal gas constant ($R = 8.314$ J/mol K) [9]. Inserting this in Eq. (15), we get

$$\mu^a(n^a, T, A) - \mu_0^a = RT \ln \left(\frac{n^a}{n_0^a} \right). \tag{17}$$

For the protein in aqueous solution (sorptive phase), we choose the ideal osmotic model, i.e., assume the osmotic pressure of the protein to be

$$p^f = cRT = \frac{n^f}{V_0} RT. \tag{18}$$

Using the analogy: ideal gas molecule / vacuum $\simeq$ protein (particle) / water, we can write for the chemical potential of the protein:

$$\mu^f(p^f, T) = \mu^f(p_0^f, T) + RT \ln \left(\frac{p^f}{p_0^f} \right), \tag{19}$$

which, in view of Eqs. (18) and (2), can be rewritten as

$$\mu^{f}(c, T) = \mu^{f}(c_0, T) + RT \ln\left(\frac{c}{c_0}\right). \tag{19a}$$

The condition for adsorption equilibrium (10) now delivers together with the model equations for the chemical potentials of the sorptive (f) and the adsorbed (a)-phase (19a), (17) the adsorption isotherm of the system,

$$n^{a}(c, T) = K(T)c, \tag{20}$$

with the temperature-dependent constant,

$$K(T) = n_0^{a}(c_0, T)/c_0. \tag{21}$$

Here, (n_0^{a}) is the amount of protein adsorbed in the isothermal reference state of the system with bulk concentration ($c_0 = n_0^{f}/V_0$). According to Eq. (20), the amount of protein adsorbed increases linearly with the concentration of protein in the surrounding liquid phase. This equation also may be interpreted as a first-order Taylor series expansion of the sorbate function $n^{a} = n^{a}(c, T)$, higher-order terms probably being necessary at increasing protein concentrations (c) in the solution. Indeed, if the protein in the solution is not described by the chemical potential (19) but by its real fugacity (f), i.e., if

$$\mu^{f}(c, T) = \mu^{f}(c_0, T) + RT \ln\left(\frac{f(c, T)}{c_0}\right), \tag{22}$$

the equilibrium condition (10) leads to the isotherm

$$n^{a}(c, T) = K(T)f(c, T), \tag{23}$$

which, after introducing a quasi virial expansion for the fugacity,

$$f(c, T) = c(1 + B(T)c + C(T)c^2 + \ldots), \tag{24}$$

leads to a series expansion form of the adsorption isotherm (23) as

$$n^{a}(c, T) = K(T)c(1 + B(T)c + C(T)c^2 + \ldots). \tag{25}$$

Naturally, thermodynamics can neither give explicit expressions for the temperature-dependent virial coefficients B = B(T), C = C(T)..., nor can it deliver numerical values or these coefficients. They have to be determined from carefully performed experiments [D10, D11 in Part I].

4. The Langmuir adsorption isotherms (N = 1)

An adsorption isotherm which often is used to describe nonlinear effects in singl-component (N = 1) protein adsorption equilibria is that given by Langmuir (1916) [5, 8, 9]. Its main prerequisites are

- the adsorbed proteins (adproteins) form at most a single layer, i.e., no multiple layer sorbates occur;
- all adsorption sites are energetically equivalent, i.e., the energy or enthalpy of adsorption is a constant;
- the adproteins are isolated from each other, i.e., no interactions between adproteins are taken into account.

If these assumptions hold, the adsorption process of the system in Figure 1 can simply be modeled as follows: The number of proteins adsorbed during the time interval (t, t + dt) is proportional to the concentration (c) of proteins in solution and to the number of free or unoccupied adsorption sites available on the surface, given by

$$dn^{+} = k^{+}c(n_{\infty} - n^{a})dt. \tag{26}$$

Here, (n_{∞}) is the number of proteins adsorbed on the surface (A) of the sorbent material (m^{s}) in a monolayer at limiting protein concentration ($c \rightarrow \infty$) where (k^{+}) is a constant. Likewise, the number of proteins being desorbed during (t, t + dt) is proportional to the number (n^{a}) of proteins already being adsorbed:

$$dn^{-} = k^{-}n^{a}dt. \tag{27}$$

For equilibrium, the condition

$$dn^{+} = dn^{-} \tag{28}$$

must hold. Hence we get with Eqs. (26) and (27)

$$n^a(c, T) = n_\infty(T)\frac{b(T)c}{1 + b(T)c}, \quad b = k^+/k^-, \tag{29}$$

with the limiting condition

$$\lim_{c\to 0} n^a(c, T) \simeq n_\infty(T)b(T) \cdot c, \tag{29a}$$

$$\lim_{c\to \infty} n^a(c, T) \simeq n_\infty(T). \tag{29b}$$

The parameters $n_\infty(T)$, $b(T)$ may depend on the temperature of the system. Indeed, simple functions for these are, cf. also Eq. (D63) in Part I,

$$n_\infty(T) = n_\infty(T_0)\ \exp\left(-\frac{Q_s}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)\right), \tag{30}$$

$$\frac{1}{b(T)} = \frac{1}{b(T_0)}\ \cdot \exp\left(-\frac{Q_a}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)\right). \tag{31}$$

Here (Q_s) is a normally very small energy related to the thermal expansion/contraction process of the sorbent material and $(n_\infty(T_0))$ is the limiting amount adsorbed in a monolayer at the surface of the sorbent at the reference temperature (T_0). Likewise, (Q_a) is the adsorption enthalpy at (T_0) and $(b(T_0))^{-1}$ is the protein concentration in the solution needed to get the "half-load" of (n_∞) to be adsorbed, i.e., we have $n^a(b^{-1}(T_0), T_0) = n_\infty/2$.

By inversion of Eq. (29), we can also simply get the so-called adsorption isostere, i.e., the concentration of protein needed in the liquid sorptive phase to maintain a given amount of protein (n^a) to be adsorbed:

$$c\ (n^a, T) = \frac{1}{b\ (T)} \cdot \frac{n^a/n_\infty}{1 - (n^a/n_\infty)}. \tag{32}$$

4.1. Kinetics of the Langmuirian adsorption process

The kinetics of the protein adsorption process related to the model equations (26) and (27) can be easily presented by using the balance equation for the protein adsorbed:

$$dn^a = dn^+ - dn^-. \tag{33}$$

This, together with Eqs. (26) and (27), leads for constant bulk concentration ($c = \text{const}$) of the protein and the initial condition $n^a(0) = 0$ after simple integration to

$$n^a(t) = n_\infty \frac{b\,c}{1 + b\,c}\,(1 - e^{t/\tau}), \tag{34}$$

$$\frac{1}{\tau} = k^-(1 + b\,c), \tag{35}$$

i.e., a simple relaxation behavior of the amount of protein adsorbed limiting in its equilibrium value already given by Eq. (29). The relaxation time (τ) is according to Eq. (35) the shorter, the higher the bulk concentration (c) in the solution is.

If however c varies in time, the foregoing result is not valid. Instead, one has to consider a more reliable kinetic theory of the adsorption/desorption process. A simple example for this is provided by thermodynamics of irreversible processes, cf. literature given in Part I [E3, E4, D7, D12]. To elucidate this formalism, we start by considering the adsorbed proteins (adsorbate) as an open thermodynamic system exchanging mass with its bulk solution surroundings and being at isothermal conditions with constant sorptive active surface (A). Then, from the

– First Law of Thermodynamics:

$$dU^a = dQ + h^f\,dn^a, \tag{36}$$

– the Gibbs equation:

$$dS^a = \frac{1}{T}dU^a + \frac{\pi}{T}dA - \frac{\mu^a}{T}dn^a, \tag{37}$$

– and the balance equation for the entropy:

$$dS^a = \frac{dQ}{T} + s^f\,dn^a + dS^a_{in}, \tag{38}$$

the entropy production of the adsorption process can be derived as

$$P_s = \left(\frac{d\,S^a_{in}}{dt}\right)_T = \frac{1}{T}(\mu^f - \mu^a)\,\dot{n}^a \;\geq\; 0. \tag{39}$$

According to the principle of Eckart and Onsager, a process equation for the "thermodynamic flux" ($\dot{n}^a$) can be set up as a series expansion in the "thermodynamic force" ($\mu^f - \mu^a$), i.e.,

$$\dot{n}^a(t) = L_p A(\mu^f(c, T) - \mu^a(n^a, A, T)) + O(2). \tag{40}$$

In the equations above, (U^a, Q, h^f) indicate the internal energy of the adsorbate, the heat exchanged during the adsorption process, and the molar enthalpy of the proteins coming in from the bulk phase and being adsorbed. Similarly, (S^a, s^f, S^a_{in}) are the entropy of the adsorbate, the molar entropy of the proteins in the bulk phase, and the entropy produced during the adsorption process. Finally, (L_p) is a phenomenological mass transfer coefficient characterizing the adsorption process. According to the 2nd Law (39), it always is positive ($L_p \geq 0$).

Now, for the sake of simplicity, we restrict the discussion to ideal protein solutions, i.e., we assume for the chemical potential the form

$$\mu^f(c, T) = \mu^f(c^+, T) + RT \ln\left(\frac{c}{c^+}\right). \tag{41}$$

Here, (c^+) is the protein concentration of a certain reference state. In view of the equilibrium condition for the sorption system (10), we then can write for the chemical potential of the protein adsorbate using Eq. (41) and the substitution ($c^+ \to c$, $c \to c_E(n^a, A, T)$):

$$\begin{aligned}\mu^a(n^a, A, T) &= \mu^f(c_E(n^a, A, T), T) \\ &= \mu^f(c, T) + RT \ln\left(\frac{c_E(n^n, A, T)}{c}\right).\end{aligned} \tag{42}$$

Here, (c_E) indicates the fluid concentration of the protein that would be necessary to equilibrate the protein adsorbate at amount (n^a), sorption active surface (A), and temperature (T). Assuming the adsorbate to obey the Langmuir isotherm (29), (c_E) is given as a function of (n^a) by Eq. (32). Hence, we get from the process equation (40) together with Eqs. (42), (32):

$$\dot{n}^a(t) + L_p A\ RT\ \ln\left(\frac{n^a/n^a_\infty}{b(1 - (n^a/n^a_\infty)}\right) = LART\ \ln c(t), \tag{43}$$

which is an ordinary differential equation (ODE) for the amount of protein adsorbed ($n^a(t)$) at the given (and possibly time-dependent) protein bulk concentration (c(t)). For its solution, an initial condition ($n^a(t = 0) = n^a_0$) has to

be specified and numerical methods (MATH-CAD etc.) have to be applied. As adsorption processes of proteins are gaining increasing application, primarily for protein isolation and purification, it would be worthwhile to apply the above formalism to a real protein adsorption process. A thesis dealing with the adsorption of bovine-α-lactalbumin on a polymer is in preparation and should be available in 2010.

4.2. Multicomponent protein adsorption

Let us now extend the formalism given above to biofluids including not only one but many different types of proteins, enzymes, etc., all of which have a certain tendency to adsorb on the surface of a given sorbent. That is, we are now going to consider coadsorption processes, which often can be found in real biofluids or broths. The system consists of an inert sorbent of mass (m^s), sorption active surface (A), liquid water as solvent (n_w), and $N \geq 1$ different proteins of total mole numbers:

$$n_{io} = n_i^f + n_i^a, \quad i = 1 \ldots N. \tag{44}$$

Here, (n_i^a) is the number of moles of protein (i) adsorbed on (A) and (n_i^f) is the number of moles of protein (i) still in the aqueous solution. All phases are at the same temperature (T). The external pressure is (p). The system is sketched in Figure 2.

The molar concentration of the proteins in the solution are

$$c_i = \frac{n_i^f}{V^f} = y_i c, \quad i = 1 \ldots N., \tag{45}$$

with the volume of the solution,

$$V^f = V^f(n_w, n_1^f \ldots n_N^f, p, T), \tag{46}$$

and the molar fractions (y_i), total mole number of proteins in solution (n^f), and total concentration (c) defined by

$$y_i = \frac{n_i^f}{n^f} = \frac{c_i}{c}, \quad i = 1 \ldots N, \tag{47}$$

$$n^f = \sum_{i=1}^{N} n_i^f, \tag{48}$$

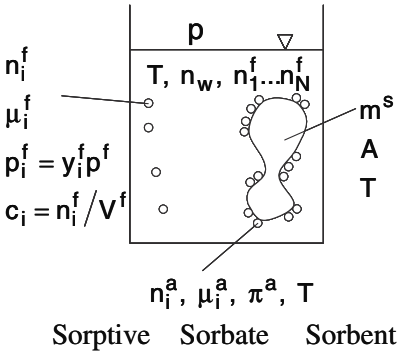

Figure 2 Multi-component sorption system consisting of water (n_w) as solvent, $N \geq 1$ many different protein solutes (n_i^f, $i = 1 \ldots N$), a coadsorbed phase with mole numbers (n_i^a, $i = 1 \ldots N$), and an inert sorbent of mass (m^s) and surface active area (A).

$$c = \sum_{i=1}^{N} c_i. \tag{49}$$

Mind that the molar fractions (y_i) do not include the mole number of the solvent (n_w).

It is sometimes more convenient to use instead of the protein molar concentrations (c_i) the related ideal osmotic partial pressures:

$$p_i^f = \frac{n_i^f RT}{V^f} = c_i RT, \quad i = 1 \ldots N. \tag{50}$$

Obviously, in view of Eqs. (47), (49), these also can be written as

$$p_i^f = y_i p^f, \quad i = 1 \ldots N, \tag{51}$$

$$p^f = \frac{n^f RT}{V^f} = cRT, \quad i = 1 \ldots N. \tag{52}$$

The conditions for thermodynamic equilibrium between the liquid sorptive phase ($n_1^f \ldots n_N^f$) and the adsorbate phase ($n_1^a \ldots n_N^a$) can be derived from basic thermodynamic principles in the same way as for single protein solutions ($N = 1$). This has been outlined at the beginning of this section. Hence, we can restrict the discussion to citing the resulting equations, cf. Eq. (10), [12],

$$\mu_i^a(n_1^a \ldots n_N^a, A, T) = \mu_i^f(c_1 \ldots c_N, T) \quad i = 1 \ldots N, \tag{53}$$

which require equality of the chemical potentials, i.e., the tendency of a protein of component (i) to spread out from its respective phase (a, f). These equations together with the conservation equations (44) allow us in principle to calculate the distribution of the proteins between the solution and the adsorbate phase, i.e., figures of (n_i^f, n_i^a, i = 1 ... N), if the total amounts (n_{io}) of proteins are given and models for (n_i^a, n_i^f) have been introduced.

For the liquid phase, we restrict the discussion here to presenting the (formal) representation of the chemical potential by its mixture fugacities [E5 in Part 1], [12], i.e.,

$$\mu_i^f(c_1 \ldots c_N, T) = \mu_{i0}(c_{i0}, T) + RT \ \ln\left(f_i(c_1 \ldots c_N, T)/c_{i0}\right), \tag{54}$$

with the Taylor series expansion,

$$f_i(c_1 \ldots c_N, T) = c_i \left(1 + \sum_{k=1}^{N} B_{ik}(T)\, c_k + \sum_{k=1}^{N} C_{ikl}(T)\, c_k c_l + \ldots\right), \tag{55}$$

and (c_{i0}) being a reference concentration that may be chosen for any component individually. Also in Eq. (54) (μ_{io}) is the chemical potential of the pure protein (i) in the aqueous solution at concentration (c_{i0}) and temperature (T). It should be noted that in principle the fugacity (f_i) also would depend on the choice of the reference concentration (c_{i0}) and on the system's pressure (p). However, in engineering applications both dependencies are neglected for the sake of simplicity or – more often – time and economic pressures.

As far as the coadsorbate ($n_1^a \ldots n_N^a$) is concerned, we restrict ourselves to mentioning only

– the multicomponent extension of the ideal adsorption isotherm in its virial expansion (25), which reads as

$$n_i^a(c_1 \ldots c_N, T) = K_i(t)c_i \left(1 + \sum_{k=1}^{N} B_{ik}(T)c_k + \sum_{k,l=1}^{N} C_{ikl}(T)c_k c_l + \ldots\right), \tag{56}$$

with

$$K_i(T) = \frac{n_i^a(c_{io}, T)}{c_{io}}. \tag{57}$$

Here ($c_{i0} \to 0$) is a small reference concentration and ($n_i^a(c_{io}, T)$) the respective amount adsorbed at this concentration. The temperature-dependent virial coefficients ($B_{ik}(T)$, $C_{ikl}(T)\ldots$) are defined by the series expansion of the fugacity of protein (i) in the liquid phase, cf. Eq. (55). These coefficients in principle can be experimentally determined by osmotic pressure measurements [13]. If the series expansion in Eq. (56) is truncated after the zeroth order term, the resulting isotherms,

$$n_i^a(c_1 \ldots c_N, T) = K_i(T)c_i, \quad i = 1 \ldots N, \tag{58}$$

obviously only hold for a very small concentration, i.e., in the so-called Henry limit ($c_i \to 0$).

– Also we would like to mention the multi-component extension of the Langmuir adsorption isotherm (29). It is based on the two-model equation for the amount of protein of type (i) being adsorbed (dn^+) and desorbed (dn^-), respectively, within a time interval (dt):

$$dn_i^+ = k_i^+ c_i \left(n_\infty - \sum_{k=1}^{N} n_k^a \right) dt, \quad i = 1 \ldots N, \tag{59}$$

$$dn_i^- = k_i^- n_i^a dt. \tag{60}$$

The dynamic equilibrium conditions

$$dn_i^+ = dn_i^-, \quad i = 1 \ldots N, \tag{61}$$

deliver together with Eqs. (59), (60) the isotherms

$$n_i^a(c_1 \ldots c_N, T) = n_\infty(T) \frac{b_i(T)c_i}{1 + \sum_{k=1}^{N} b_k(T)c_k}, \quad i = 1 \ldots N, \tag{62}$$

$$b_i(T) = \frac{k_i^+}{k_i^-}. \tag{63}$$

The isotherm (62) has the limiting properties

$$\lim_{c_i \to \infty} n_i^a = n_\infty(T), \quad i = 1 \ldots N, \tag{64}$$

$$\lim_{c_i \to 0} n_i^a \cong \frac{b_i n_\infty}{1 + \sum_{k \neq 1}^{N} b_k c_k} c_i. \tag{65}$$

Several generalizations of the isotherms (62) referring most often to the structure of the sorbent surface (A), i.e., its fractality, pore spectrum, etc.. are known in the literature [5]. A special case of this will be discussed in the next section of this chapter. Finally we denote the adsorption isosteres referring to the isotherms (62). These can be easily calculated by applying symmetry arguments. We get, cf. Eq. (32),

$$c_i(n_1^a \ldots n_N^a, T) = \frac{1}{b_i(T)} \cdot \frac{n_i^a}{n_\infty} \cdot \frac{1}{1 - \sum_{k=1}^{N} n_k^a / n_\infty} \quad i = 1 \ldots N. \tag{66}$$

These equations allow the calculation of the protein concentrations (c_i) necessary in the fluid phase to maintain the adsorbate loads ($n_1^a \ldots n_N^a$).

The kinetics of pure coadsorption processes at constant bulk concentrations ($c_1 \ldots c_N$) and given initial conditions ($n_i^a(t = 0) = n_{i0}^a, i = 1 \ldots N$) can be easily obtained from the model equations (59), (61) and the protein adsorbate balance equations:

$$dn_i^a = dn_i^+ - dn_i^-, \quad i = 1 \ldots N. \tag{67}$$

The result is

$$\dot{n}_i^a + b_i c_i \sum_{k=1}^{N} n_k^a = b_i c_i n_\infty = \text{const}, \quad i = 1 \ldots N. \tag{68}$$

These are (N) many ordinary differential equations (ODEs) for the functions ($n_1^a(t) \ldots n_N^a(t)$). Depending on the eigenvalues of the respective matrix, oscillations reflecting replacement adsorption between different components may also occur in coadsorption relaxation. More details about this will be given in another paper.

For a description of coadsorption processes occurring in bulk protein solutions with concentrations varying in time ($c_1(t) \ldots c_N(t)$), the method of thermodynamics of irreversible processes is again recommended, cf. [D12, D13 in Part 1]. We restrict ourselves here to presenting the main results, cf.

Eqs. (36)–(40): The entropy production in the (isothermal) system in Figure 2 during a coadsorption process is

$$P_s = \left(\frac{dS^a_{in}}{dt}\right)_T = \frac{1}{T}\sum_{i=1}^{N}(\mu_i^f - \mu_i^a)\,\dot{\mu}_i^a \geq 0, \tag{69}$$

with "thermodynamic forces" $(\mu_i^f - \mu_i^a)$ and "thermodynamic fluxes" $(\dot{\mu}_i^a)$, $i = 1\ldots N$. Accordingly, we assume the process equations, i.e., relations between the forces and fluxes in linear approximation, to have the structure:

$$\dot{n}_i^a(t) = A\sum_{k=1}^{N} L_{ik}(\mu_k^f(c_1\ldots,T) - \mu_k^a(n_1\ldots,T)), \quad i = 1\ldots N. \tag{70}$$

Here the phenomenological coefficients $(L_{ik}, i, k, = 1\ldots N)$ describe for $i \neq k$ cross-effects between different types of proteins possibly interfering with each other during the coadsorption process. The matrix (L_{ik}) is symmetrical, i.e., the Onsager relations,

$$L_{ik} = L_{ki}, \quad i,k = 1\ldots N, \tag{71}$$

hold. Also, the matrix (L_{ik}) is positive definite [5], according to the Second Law (69):

$$\|L_{ik}\| \geq 0,\ L_{ii} \geq 0,\ L_{ii}L_{kk} - L_{ik}L_{ki} \geq 0\ldots \text{etc.} \quad i,k = 1\ldots N. \tag{72}$$

The coefficients (L_{ik}) are functions of the temperature, pressure, and in principle also of the chemical composition of the sorbent fluid. They basically have to be determined experimentally, which in principle could be done by inverse reasoning of spectroscopic measurements of time-dependent adsorption loads $(n_1^a(t)\ldots n_N^a(t))$, cf. Eq. (75) and [14–16].

To transform the process equations (70) into a set of ordinary differential equations (ODEs) for the adsorbate load, we restrict ourselves to an ideal sorptive fluid phase, i.e., we assume for the chemical potentials of the proteins in the solution the truncated form, cf. Eqs. (54) and (55),

$$\mu_i^f(c_1\ldots c_N,T) = \mu_{i0}^f(c_{i0},T) + RT\ln\left(\frac{c_i}{c_{i0}}\right) \quad i = 1\ldots N. \tag{73}$$

The chemical potentials of the adsorbed proteins can be written in view of the equilibrium conditions (53) and (73) as

$$\mu_i^a(n_1^a \ldots n_N^a, , A, T) = \mu_i^f(c_{1E} \ldots (c_{NE}, T) = \mu_{i0}^f(c_{i0}, T) + RT \ln\left(\frac{c_{iE}}{c_{i0}}\right). \quad (74)$$

Here, $c_{iE} = c_{iE}(n_1^a \ldots n_N^a, T), i = 1 \ldots N$ indicate the concentrations in the liquid bulk phase that would be necessary to maintain the adsorbate loads $(n_1^a \ldots n_N^a)$ at temperature (T) in equilibrium with the fluid phase.

Introducing Eqs. (73), (74) in the process equations (70), we get in view of the isosteres (66)

$$\dot{n}_i^a(t) + ART \sum_{k=1}^{N} L_{ik} \ln\left(\frac{n_k^a}{b_k n_\infty \left(1 - \sum_{l=1}^{N} (n_l^a/n_\infty)\right)}\right) = ART \sum_{k=1}^{N} L_{ik} \ln c_k, \quad (75)$$

$i = 1 \ldots N.$

This is a set of (N) ODEs from which the adsorbate loads $(n_1(t) \ldots n_N(t))$ can be calculated if the bulk concentrations $(c_1(t). . . c_N(t))$ of the proteins in the fluid phase are known as functions of time, and initial conditions $(n_i(t = 0) = n_{io}, i = 1. . .N)$ of the preadsorbed proteins are given. For the solution, implicit numerical methods are recommended to assure stability of calculations.

5. Multi-contact adsorption of proteins from solutions

Proteins normally have many surface-active atomic groups on their surface, i.e., spots attracted by other atoms of a solid surface. Hence it is to be expected that adsorption of a protein often will occur not only at one but at several atomic group contacts, thus intensifying the interaction between the protein and the sorbent surface. Conversely, desorption will become more difficult, as for this not only one but all contacts between the protein's surface atoms and the sorbent atoms have to be lifted [10, 11]. Phenomenologically, this can easily be described by a modified Langmuirian adsorption isotherm as follows. We consider again the system depicted in Figure 1 and restrict ourselves to mono-layer adsorption phenomena. Assuming a single protein to adsorb not only at one site but at $a \geq 1$ many energetically equivalent sites, the number of proteins adsorbing in a time element (dt) is given by

$$dn^+ = k^+ c(n_\infty - a \cdot n^a)dt, \quad (76)$$

whereas the number of proteins desorbing in (dt) is

$$dn^{-} = \alpha(a) k^{-} n^{a} dt. \tag{77}$$

Here ($k^{\pm}$) are characteristic constants referring to the single site ($a = 1$) adsorption and desorption process, cf. Eqs. (26) and (27), (c) is the bulk concentration of the protein in the solution in (mol/l) or (μ mol/l), (n_{∞}) is the number of adsorption sites, and (n^{a}) is the number of proteins already adsorbed at time (t) on the sorption active surface (A) of the sorbent. The protein-dependent function $\alpha = \alpha(a) \leq 1$ is a reduction factor of the desorption process. In case all contact sites are independent of each other and also related to the same desorption energy, $\alpha(a)$ is by purely combinational arguments given by

$$\alpha(a) = \frac{1}{2^{a-1}} < 1, \quad a \geq 1. \tag{78}$$

The equilibrium condition

$$dn^{+} = dn^{-} \tag{79}$$

delivers with Eqs. (76) and (77) the isotherm

$$n^{a}(c, A \simeq m^{s}, T) = n_{\infty} A T \frac{b_{a}(T) c}{1 + a\, b_{a}(T) c} \tag{80}$$

with

$$b_{a}(T) = \frac{b_{1}(T)}{\alpha(a)}, \quad b = \frac{k^{+}}{k^{-}} \tag{81}$$

and the limiting relations

$$\lim_{c \to 0} n^{a}(c, T) \simeq b_{a} n_{\infty} c > b_{1} n_{\infty} c, \tag{82}$$

$$\lim_{c \to \infty} n^{a}(c, T) = \frac{n_{\infty}}{a} < n_{\infty}. \tag{83}$$

That is, the multi-contact effects lead at very low concentration to higher adsorption loads compared to that in the case of single contacts but to a lower asymptotic load approached at high protein concentrations in the bulk sorptive phase. This situation is sketched in dimensionless variables in Figure 3.

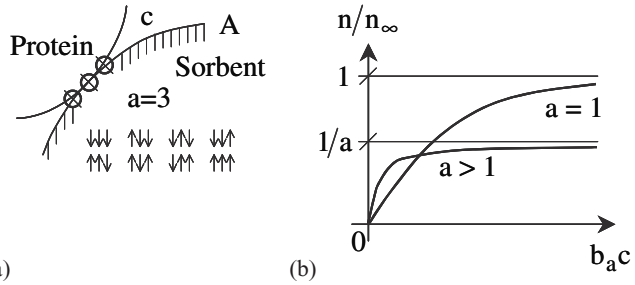

Figure 3 (a) Multi-contact adsorption of a protein on an open sorbent surface (A) with number of adsorption contacts ($a = 3$). (b) Qualitative behavior of the adsorption isotherm (80) for single adsorption contact ($a = 1$) and multi-contact adsorption ($a > 1$).

The number of contacts (a) and the desorption reduction function $\alpha(a)$ on principle can be determined from isotherm measurements, i.e., Eqs. (82), (83), if a reference substance is at hand which exhibits single contact adsorption, i.e., would allow the determination of the parameters (n_∞, b_1), which generally are expected to depend on the temperature (T) of the bulk solution.

The formalism presented above can easily be extended to multi-component protein solutions, with each component showing multi-contact adsorption. This situation is sketched for two proteins (i, k) in Figure 4. By analogous reasoning, we can model the number of proteins of type (i) adsorbed (dn_i^+) to or desorbed (dn_i^-) from the surface (A) by the relations, cf. Eqs. (76) and (77), as

$$dn_i^+ = k_i^+ c_i (n_\infty - \sum_{k=1}^{N} a_k n_k^a) dt, \tag{84}$$

$$dn_i^- = \alpha_i(a_i)\, k_i^-\, n_i^a dt, \; i = 1 \ldots N. \tag{85}$$

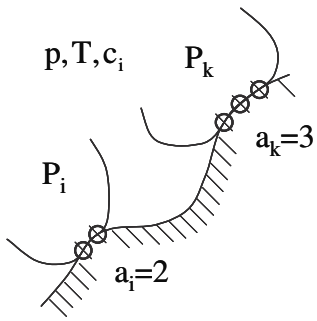

Figure 4 Multi-contact coadsorption of proteins of different types (P_i, P_k) with contact numbers ($a_i = 2$, $a_k = 3$) on an open sorbent surface (A).

Here the parameters ($k_i^{\pm}, i = 1 \ldots N$) refer to single site adsorption and desorption processes ($a_i = 1$) of type (i), (c_i) is the bulk concentration of protein (i), ($n\infty$) is the number of adsorption contact sites available on the surface (A) of the sorbent, and $\alpha_i = \alpha_i(a_i)$ are desorption reduction functions of the proteins, which basically have to be measured but may be guessed from combinatorial arguments such as

$$\alpha_i(a_i) = \frac{1}{2^{a_i - 1}} < 1, \quad a_i \geq 1 \quad i = 1 \ldots N. \tag{86}$$

The equilibrium condition for protein (i),

$$dn_i^+ = dn_i^- \tag{87}$$

leads together with Eqs. (84), (85) to the isotherm

$$n_i^a(c_1 \ldots c_N, A \simeq m^s, T) = n_\infty(A, T) \frac{b_{ai}(T)\, c_i}{1 + \sum_{k=1}^{N} a_k b_{ak}(T) c_k}, \tag{88}$$

$$i = 1 \ldots N$$

with

$$b_{ai}(T) = \frac{b_i(T)}{\alpha_i(a_i)}, \quad b_i(T) = \frac{k_i^+}{k_i^-} \tag{89}$$

and the limiting relations

$$\lim_{c_i \to 0} n_a^i(c_1 \ldots c_N, T) \simeq \frac{n_\infty b_{ai}}{1 + \sum_{k \neq i}^{N} a_k b_{ak} c_k} c_i \quad i = 1 \ldots N. \tag{90}$$

According to Eq. (90), the coadsorption isotherm of (n_a^i) at low concentrations (c_i) can be very different from that of pure component adsorption (82), i.e., it can be either steeper or flatter, reflecting the influence of other components in the adsorbate. Naturally, all the parameters in Eqs. (88)–(89) have to be determined from pure component adsorption measurements at low bulk concentrations (c_i). For higher concentrations, mixture effects in the bulk phase should be taken into account, cf. the osmotic virial expansion (55).

6. Adsorption-induced unfolding of proteins

6.1. Basic thermodynamic formalism

Proteins may change their higher order structure upon adsorption, i.e., the enzymatic, physiological, and biosensoric properties of the protein are sensitive to its interactions with open or porous solid surfaces. As an example, we only mention the denaturation process of bovine α-lactalbumin, which in bulk solutions at ambient conditions and pH = 8 has half-times of about 10^3 s. Upon adsorption on (inert) polystyrene nanospheres of diameter (d ≃ 100 nm), the half-time for denaturation is reduced to only 0.1 s, i.e., by 4 orders of magnitude [17].

In this section, we are going to outline a formalism to describe this phenomenon, i.e., the interaction between a denaturation process of a protein in a bulk solution, the adsorption process of the protein on a solid surface, and the denaturation process of the protein occurring in the adsorbed state. A scheme of this situation is sketched in Figure 5.

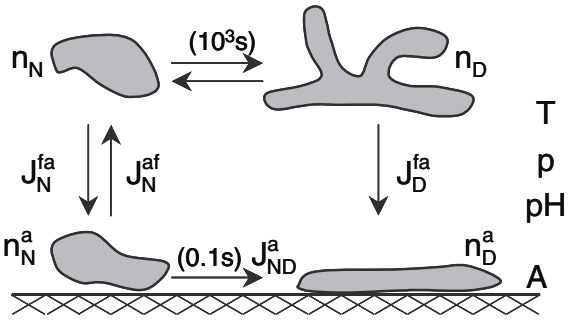

Figure 5 Protein solution including a sorption active surface (A). The protein in the solution is in either its native state (n_N) or denatured or unfolded state (n_D). The protein also may adsorb on (A) and then either stay in its native state (n_N^a) or turn to its denatured state (n_D^a).

The various occupation numbers ([n] = mol) and fluxes ([J] = mol/s) have the following meaning:

n_N ... protein in native state (N) dissolved in the bulk solution,
n_D ... protein in denatured state (D) dissolved in the bulk solution,
n_N^a ... protein in native state adsorbed on the surface (A) of the sorbent material,
n_D^a ... protein in denatured state (D) adsorbed on the surface (A),
J_N^{af} ... flow of proteins in native state from the bulk fluid phase (f) to the adsorbed phase (a),

J_D^{fa} ... flux of protein in denatured state from the bulk fluid phase to the adsorbed phase (adsorbate),

J_{ND}^{a} ... transition rate of protein in native state (N) to the denatured state (D) within the adsorbed phase.

The symbols (T, p, pH) indicate the temperature, pressure, and pH value of the solution.

To set up the model, we first consider the molar balance equations for the occupation numbers (n_N, n_D, n_N^a, n_D^a):

$$\dot{n}_N = -\dot{\xi}_{ND} - J_N^{fa} + J_N^{af}, \tag{91}$$

$$\dot{n}_D = -\dot{\xi}_{ND} - J_D^{fa}, \tag{92}$$

$$\dot{n}_N^a = J_N^{fa} - J_N^{af} - J_{ND}^{a}, \tag{93}$$

$$\dot{n}_D^a = J_D^{fa} + J_{ND}^{a}. \tag{94}$$

Here, ($\dot{\xi}_{ND}$) is the rate of the transition process of the protein from its native to a denatured state (N $\rightarrow$ D) or, possibly, to a whole set of different states of denatured type. We assume here the transition process to be a quasi-chemical reaction. Then ($\dot{\xi}_{ND}$) can be modeled by thermodynamics of irreversible processes in the linear approximation as (cf. chapter "Denaturation of Proteins" in a subsequent paper)

$$\dot{\xi}_{ND} = A_{ND}(\mu_N - \mu_D). \tag{95}$$

In this equation, (μ_N, μ_D) indicate the chemical potentials (kJ/mol) of the protein in the solution in its native (N) and denatured state (D), respectively. Also, ($A_{ND} > 0$) is a phenomenological transition parameter that actually has to be determined experimentally, i.e., by measuring the concentration of the protein in its native (N) and denatured state (D):

$$c_N(t) = \frac{n_N(t)}{V^f}, \quad c_D(t) = \frac{n_D(t)}{V^f}, \tag{96}$$

$V^f = V^f(p, T, n_w, n_p = n_N + n_D)$ being the volume of the bulk solution.

Equation (95) can be rewritten as

$$\dot{\xi}_{ND} = A_{ND} RT \ln\left(\frac{y_N y_{DE}}{y_{NE} y_D}\right). \tag{97}$$

Here the molar fractions,

$$y_N = \frac{c_N}{c_N + c_D}, \quad y_D = \frac{c_D}{c_N + c_D}, \tag{98}$$

and the equilibrium concentrations,

$$y_{NE} = \frac{n_{NE}}{n_{NE} + n_{DE}}, \quad y_{DE} = \frac{n_{DE}}{n_{NE} + n_{DE}}, \tag{99}$$

belonging to the same total amount of protein included in the solution

$$n = n_N + n_D \tag{100}$$

have been introduced. These concentrations are given via the law of mass action, cf. Chapter "Denaturation of Proteins", in the "ideal approximation" as

$$\frac{y_{NE}}{y_{DE}} = \exp\left(-\frac{\Delta G_{ND}}{RT}\right) \tag{101}$$

with

$$\Delta G_{ND} = \mu_{N0}(c, T) - \mu_{D0}(c, T), \tag{102}$$

$$c = \frac{n_N + n_D}{V^f} = \frac{n}{V^f}. \tag{103}$$

This quantity also has to be determined experimentally as for example by micro-calorimetric measurements leading to the (free) enthalpy of the transition ($N \leftrightarrow D$).

Obviously, the term on the r. h. s. of Eq. (97) describes the deviation from equilibrium of the transition reaction ($N \leftrightarrow D$), i.e., the term vanishes for ($y_N = y_{NE}, y_D = y_{DE}$).

The fluxes (J_N^{fa}, J_N^{af}) in Eq. (91) are modeled in the Langmuir style, i.e., we have, cf. Eqs. (26), (59):

$$J_N^{fa} = k_N^+(n_\infty - n_N^a - n_D^a)c_N, \tag{104}$$

$$J_N^{af} = k_N^- n_N^a, \tag{105}$$

with (k_N^+, k_N^-) being two phenomenological coefficients describing adsorption to and desorption from the sorbent surface and depending mostly on the interaction between the native protein and the sorbent, i.e., the adsorption energy, which may be in the range (20–1000) kJ/mol.

The flow of denatured protein to the sorbent surface is also modeled by a Langmuirian equation as

$$J_D^{fa} = k_D^+(n_\infty - n_N^a - n_D^a)c_D, \tag{106}$$

with the phenomenological transition parameter (k_D^+).

For the transition of the protein in the adsorbate from its native (N) to the denatured state (D), we assume the process to be similar to the radioactive decay, i.e., proportional to the amount of protein adsorbed on (A) in its native state

$$J_{ND}^a = \frac{1}{\tau_{ND}^a} n_N^a \tag{107}$$

with a protein–sorbent system characteristic decay time (τ_{ND}^a), which may range from (μs) to hours or days.

Mind that refolding of the protein (D $\rightarrow$ N) in the adsorbate as well as desorption of the denatured protein have been neglected in the model for the sake of simplicity. Inserting now all the model equations for the fluxes $(J_N^{fa} \ldots)$ into the balance equations for (n_N, n_D, n_N^a, n_D^a), we get four ordinary differential equations (ODEs), which together with initial conditions (t = 0),

$$n_N(0) = n_{N0}, n_D(0) = n_{D0}, n_N^a(0) = n_{N0}^a, n_D^a(0) = n_{D0}^a, \tag{108}$$

and the conservation equation of the protein,

$$n_N(t) + n_D(t) + n_N^a(t) + n_D^a(t) = n_0 = \text{const} \tag{109}$$

or

$$\dot{n}_N + \dot{n}_D + \dot{n}_N^a + \dot{n}_D^a = 0, \tag{110}$$

can be solved numerically if all process parameters included, namely

$$V^f,\ A_{ND},\ \Delta G_{ND},\ k_N^+,\ k_N^-,\ k_D^+,\ \tau_{ND}^a, \tag{111}$$

are known – or reasonably guessed by experience.

The resulting ordinary differential equations are:

$$\dot{n}_N + A_{ND}\,RT\,\ln\left(\frac{y_N y_{DE}}{y_{NE} y_D}\right) - (k_N^+ c_N + k_N^-) n_N^a - k_N^+ c_N n_D^a + k_N^+ n_\infty = 0, \quad (112)$$

$$\dot{n}_D + A_{ND}\,RT\,\ln\left(\frac{y_N y_{DE}}{y_{NE} y_D}\right) - k_D^+\, c_D\, n_N^a - k_D^+ c_D\, n_D^a + k_D^+ n_\infty = 0, \quad (113)$$

$$\dot{n}_N^a + \left(k_N^+ c_N + k_N^- - \frac{1}{\tau_{ND}^a}\right)\, n_N^a - k_N^+\, c_N\, n_D^a + k_N^+ c_N\, n_\infty = 0, \quad (114)$$

$$\dot{n}_D^a + \left(k_D^+ c_D - \frac{1}{\tau_{ND}^a}\right) n_N^a + k_D^+ c_D\, n_D^a - k_D^+ n_\infty = 0. \quad (115)$$

According to the structure of these equations, we expect them to be for reasonable numerical values of their parameters (110) numerically stiff [18]. Hence, implicit or soft methods are recommended for the solution [19]. Obviously, due to the decay law (107), the asymptotic state of the system for ($t \to \infty$) will be accumulation of denatured protein in the adsorbate phase. If this is not observed in experiments, the model should not be used as it must be modified to take into account this and possibly other empirical facts and properties.

As a simple approximation to the denaturation processes sketched in Figure 5, one may neglect in a first step the denaturation of the protein in the bulk liquid phase (f), cf. Figure 6.

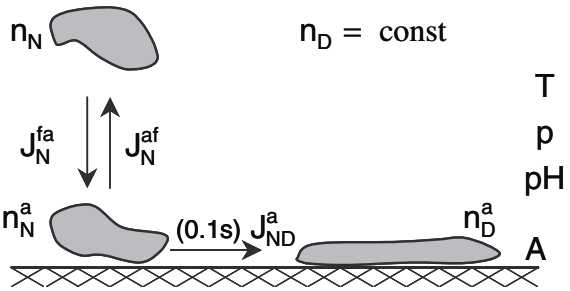

Figure 6 Simple model for denaturation of a protein after adsorption on a solid surface. The sorption process most easily can be modeled by Langmuirian equations (104), (105). The denaturation process can be approximated by a stochastic decay model similar to the radioactive decay of atoms (107).

This seems to be justified as this process is often much slower than both the adsorption process of the protein and the denaturation or decay of the protein

in the adsorbed phase (a). Taking into account only the latter two processes, i.e., assuming ($A_{ND} = 0$, $J_D^{fa} = 0$, or $k_D^+ = 0$, $n_D = \text{const}$), Eqs. (112), (114), and (115) reduce to

$$\dot{n}_D - (k_N^+ c_N + k_N^-) n_N^a - k_N^+ \, c_N \, n_D^a + k_N^+ \, n_\infty \, c_N = 0 \tag{112a}$$

$$\dot{n}_N^a + \left(k_N^+ \, c_N \frac{1}{\tau_{ND}^a} \right) \, n_N^a - k_N^+ \, c_N \, n_D^a - k_N^+ \, c_N \, n_\infty = 0 \tag{114a}$$

$$\dot{n}_D^a - \frac{1}{\tau_{ND}^a} \, n_N^a = 0 \tag{115a}$$

with

$$c_N = \frac{n_N}{V^f}$$

and the free parameters

$$V^f, k_N^+, k_N^-, \tau_{ND}^a, n = n_N + n_N^a + n_D^a = \text{const.}$$

This system of three nonlinear ordinary differential equations (ODEs) may have oscillating solutions, which of course finally will lead to the asymptotic state ($t \to \infty$) where all of the protein is in the denatured adsorbed state.

Naturally, the above formalism can be expanded or generalized to multi-protein systems showing co- or competitive adsorption and possibly other interaction effects, such as regeneration of one protein by interaction with a protein of a different type in either the fluid or the adsorbate phase [20]. Analysis of these phenomena, however, is left to the younger generation of researchers.

List of symbols

A	m^2	Surface area (external and internal) of a sorbent material
$a \geq 1$	1	Number of adsorption contacts of a single protein on the surface of a sorbent material

$a_k \geq 1$	1	Number of adsorption contacts of a single protein of component (k) from a multi-component solution
b (T)	cm^3/ mol	Temperature-dependent parameter in the Langmuir adsorption isotherm, Eq. (29)
b_i	cm^3	Parameter of component (i) in the Langmuir adsorption isotherm of a multi-component adsorbate
$c = n^f / V^f$	mol / l	Molar density of protein in solution after immersion of a sorbent material in it
$c = \sum_k^N c_k$	mol / cm^3	Total molar density of all proteins in a multi-component solution
$c_0 = n_0^f/V^f$	mol / l	Molar density of protein in solution prior to immersion of a sorbent material
c_E	mol / cm^3	Equilibrium concentration of protein in a solution at a given amount (n^a) of protein being adsorbed on the surface (A) of a sorbent
$c_i = n_i^f/V^f$	mol / cm^3	Molar concentration of component (i) in solution
$f = f(p, T, c)$	mol / cm^3	Fugacity of a protein in an aqueous solution
F^a	kJ	Free energy of protein adsorbate, i.e., protein adsorbed on the surface of a sorbent material
F^f	kJ	Free energy of a protein–water solution
f_i	mol / cm^3	Fugacity of component (i) of proteins in an aqueous solution
K	cm^3	Henry constant of the Freundlich adsorption isotherm, Eq. (20)

K_i	cm^3	Henry constant of component (i) in the Freundlich adsorption isotherms of a multi-component system, cf. Eq. (58)
L_{ik}	$mol^2 / (kJ\ m^2 s)$	Phenomenological transport coefficient of a multi-component adsorption process of proteins from an aqueous solution to the surface of a sorbent material
Lp	$mol^2 / (kJ\ m^2 s)$	Phenomenological transport coefficient of an adsorption process of proteins in an aqueous solution
m^s	g	Mass of porous adsorbent
$N \geq 1$		Number of different proteins in a multi-component solution
n_i^a	mol	Number of moles of protein component (i) being adsorbed on the surface of sorbent mass (m^s)
n_i^f	mol	Number of moles of component i = 1...N in a multi-component aqueous solution of proteins after immersion of a sorbent material in it
n_0^f	mol	Mole number of protein adsorbed on the surface of the sorbent material
n_∞	mol	Limiting amount of proteins being adsorbed on the surface of a sorbent material for very high protein concentrations in the surrounding aqueous solution
n^f	mol	Mole number of protein in aqueous solution after immersion of a sorbent material in the solution
n_W	mol	Number of moles of water included in a protein–water solution
p	$Pa = Nm^{-2}$	Pressure

p^f	$Pa = Nm^{-2}$	Osmotic pressure of protein in solution
p_0^f	Pa	Osmotic pressure of protein in solution in a certain reference state
P_s	kJ / Ks	Entropy production during an adsorption process
Q_s	kJ / mol	Heat of adsorption of proteins going from an aqueous solution to the surface of a sorbent
$R = 8.314$	J / K mol	Universal gas constant
S^a	kJ / K	Entropy of the protein adsorbate
S^f	kJ / K	Entropy of protein–water solution
S_{in}^a	kJ/K	Entropy produced during an adsorption or desorption process of proteins in the protein adsorbate
T	K	Temperature
t	s	Time
T_0	K	Temperature of a reference state
U^a	kJ	Internal energy of a protein adsorbate
V_0^f	cm^3	Volume of protein–water solution prior to immersion of a sorbent material in it
V^s	cm^3	Volume of porous adsorbent
$y_i = n_i^f / \left(\sum_k^N n_k^f \right)$	1	Molar fraction of component (i) in solution
μ_i^f	kJ / mol	Chemical potential of protein component (i) of proteins in an aqueous multi-component solution
μ_i^a	kJ / mol	Chemical potential of component (i) of proteins being adsorbed on the surface of sorbent mass (m^s)

μ^a	kJ / mol	Chemical potential of proteins adsorbed on the surface of a sorbent material
μ^f	kJ / mol	Chemical potential of proteins in aqueous solution after immersion of a sorbent material in it
π	kJ / m^2	Spreading pressure of the protein adsorbed on the surface of a sorbent material
τ	s	Relaxation time of an adsorption process
ξ_{ND}	1	Extent of transition reaction of proteins going from a native state to a denatured state

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