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Quantitative Characterization of Affinity Properties of Immobilized Receptors

Vladimir M. Mirsky

1.1 Introduction

Affinity as a tendency of molecules (ligands) to associate with another type of molecules or polymers (receptor) can be described by a set of kinetic and thermodynamic parameters. These parameters include the adsorption (or binding, or association) constant, which can be recalculated as the free energy of binding, binding enthalpy and entropy, kinetic adsorption and desorption constants, and activation energies for binding and for dissociation.

There are several reasons for a quantitative characterization of affinity. The first is due to possible applications of these receptors in affinity sensors. The sensors are intended to measure *volume* concentrations of analytes. However, transducers of affinity sensors (refractometric, interferometric, mechano-acoustical, capacitive, and others) provide information on the *surface* concentration of analytes (ligands) on a layer of immobilized receptors (Figure 1.1). Therefore, it is important to obtain a calibration curve – the dependence between volume concentration of an analyte and its surface concentration. Such relations are named in physical chemistry as adsorption isotherms (the binding is usually performed at constant temperature). Several adsorption isotherms can be obtained from simple physical models. A mathematical description of these isotherms allows one not only a better understanding of binding process but also provides a mathematical basis for interpolation and extrapolation of the calibration curve, which is of importance for analytical applications.

The second reason includes material science aspects. Quantitative information can be used to make an appropriate choice of synthetic receptors for different applications and for prediction of the detection limit and selectivity of analytical devices based on these receptors. Additionally, these data can be used as descriptors for combinatorial optimization and for discovery of new sensing materials (the combinatorial approach is discussed in Chapter 3) [1–3].

The association can be investigated in the bulk phase (in the solution or suspension of the ligand and receptor molecules) or on a surface. Ligand–receptor interactions

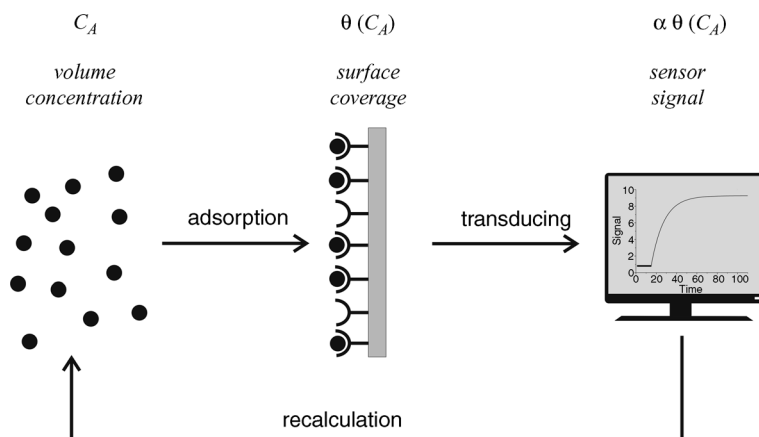


Figure 1.1 Transducers of affinity sensors provide information on the surface concentration of bound analytes. For practical applications, information on the volume concentration is required.

can be investigated in a bulk phase by titration and application of any analytical technique that is sensitive to the concentration of free or bound ligands or receptors. For example, the binding of dye molecules can be determined by colorimetry or fluorescence, while ions or redox-active species can be measured by potentiometric techniques. One of the most commonly used techniques that can be applied for various ligand–receptor systems is a NMR titration. Another general approach is based on isothermal calorimetry; in this case the heat produced during the ligand–receptor interaction is measured. This technique has the advantage of providing the enthalpy of binding in a single titration experiment. Analysis of ligand–receptor binding in bulk phases is well described in literature [4–7] and, therefore, is not covered in this chapter.

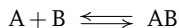
Applications of artificial receptors for chemical sensors require an immobilization of these receptors on a surface. In the 1980s to the beginning of 1990s there were many attempts to induce physical immobilization of chemoreceptors, for example, by using the Langmuir–Blodgett technique or electrostatically driven adsorption. Nowadays, receptor immobilization is performed mainly by formation of chemical bonds with some surface groups (e.g., peptide bond) or by introduction of surface anchoring groups into the receptor molecules. Chemical aspects of covalent receptor immobilization have been described [8–15]. The most widely used technology of chemical immobilization is based on the formation of an amide bond through activation by either EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide] [14, 15] or an EDC–NHS (*N*-hydroxysuccinimide) mixture. The most widely used surface anchor group is the thiol group, which forms, spontaneously, an extremely strong bond with gold, silver, palladium, copper, and some other materials [13, 16–19].

Chemical immobilization of a receptor may influence its affinity properties. This chapter is focused on the characterization of affinity properties of immobilized receptors.

1.2

Measurements Under Equilibrium Conditions

Binding of a ligand (A) with a receptor (B) leading to the formation of a complex can be considered using a formal kinetic approach:



The rate of association (adsorption onto the receptor layer) is proportional to the ligand concentration (c_L) and to the fraction of uncoated binding sites ($1 - \theta$), where θ is the fraction of occupied binding sites. Therefore, the association rate is $k_{\text{ads}}c_L(1 - \theta)$, where k_{ads} is the kinetic adsorption constant (also indicated as the kinetic association constant or kinetic binding constant). The rate of dissociation is $k_{\text{des}}\theta$, where k_{des} is the kinetic constant of desorption (also indicated as the kinetic constant of dissociation). At equilibrium conditions:

$$k_{\text{ads}} \cdot c_L(1 - \theta_{\text{eq}}) = k_{\text{des}} \cdot c_L\theta_{\text{eq}} \quad (1.1)$$

therefore:

$$\theta_{\text{eq}} = \frac{c_L K}{1 + c_L K} \quad (1.2)$$

where:

$$K = k_{\text{ads}}/k_{\text{des}} \quad (1.3)$$

This model, well known as the Langmuir adsorption isotherm, is valid for most cases of ligand–receptor binding. The model is based on the following assumptions: (i) all binding sites are equivalent; (ii) the ability of a ligand molecule to bind a binding site is independent of the occupation of neighboring sites; (iii) the number of binding sites is limited; and (iv) there is a dynamic equilibrium between bound molecules and free molecules in aqueous environment.

At low ligand concentration ($c_L K \ll 1$) this equation is linearized: $\theta = c_L K$ (Henry adsorption isotherm). Physically, it corresponds to binding at low surface coverage.

Analysis of the literature on chemical sensors, biosensors, and supramolecular chemistry demonstrates that the Langmuir adsorption isotherm is valid for most cases. The main reasons for possible deviations from this simple model are non-homogeneity of binding sites [deviation from postulate (i)] and an influence of occupation of binding sites on affinity properties of neighboring sites [deviation from postulate (ii)].

Non-homogeneity of binding sites is a typical case for artificial receptors formed by molecularly imprinted polymerization (artificial receptors based on molecularly imprinted polymers are discussed in Chapter 13) [20, 21]. For a receptor layer consisting of several different types of receptors, the Langmuir adsorption isotherm has the form:

$$\theta_{\text{eq}} = \sum_i \gamma_i \frac{c_L K_i}{1 + c_L K_i} \quad (1.4)$$

where γ_i is the molar fraction of the receptor with binding constant K_i . For a continuous distribution of binding constants the sum in Eq. (1.4) can be replaced by an integral. In practice, Eq. (1.4) can be applied only for $i = 2$ or 3 and only if the difference between K_i is at least several-fold. In some cases the heterogeneity of the receptor properties and distribution through the sensing surface can be described by fractal geometry [22]. An exponential distribution of energy of the binding sites is described by Freundlich adsorption isotherm:

$$\theta = A \sqrt[n]{c} \quad (1.5)$$

As a criterion for this isotherm, a linearization versus logarithmic concentration scale is used.

In general, although non-homogeneity of the receptor properties can be explained and described mathematically, this leads to serious practical problems in applications of such receptors for analytical purposes. In this case the results depend strongly on the initial occupation of binding states of the receptor layer, and non-complete desorption of ligands leads to a quite different adsorption isotherm.

The distance between receptors immobilized on a surface is usually large enough to exclude an influence of the occupation of one binding site on the affinity of neighboring binding sites. However, such effects have been reported in several studies. Numerous models have been suggested to describe adsorption under such conditions. The most widely used is the Frumkin isotherm.

$$Kc = \frac{\theta}{1-\theta} \exp(-2a\theta) \quad (1.6)$$

This is an extension of the Langmuir isotherm, taking into account an interaction of adsorbed molecules. The interaction is described by parameter a , which is positive for attraction and negative for repulsion between adsorbed molecules (Figure 1.2). For $a = 0$ the Frumkin isotherm coincides with the Langmuir isotherm.

A signal of affinity sensors (S) is proportional to their surface coverage (θ) (this fact is often considered as a definition of affinity sensors); therefore, for binding according to the Langmuir isotherm the signal has the following dependence on ligand concentration:

$$S = \alpha \frac{c_L K}{1 + c_L K} \quad (1.7)$$

where α is a proportionality coefficient depending on the type of transducer and on an amplification factor during the signal processing. For small concentrations ($c_L K \ll 1$) this leads to a linear equation:

$$S = \alpha c_L K \quad (1.8)$$

The main question in the analysis of experimental data obtained under quasi-equilibrium conditions is the extraction of the binding constant K . In the case of a linear dependence of the signal of an affinity sensor on concentration [Eq. (1.8)],

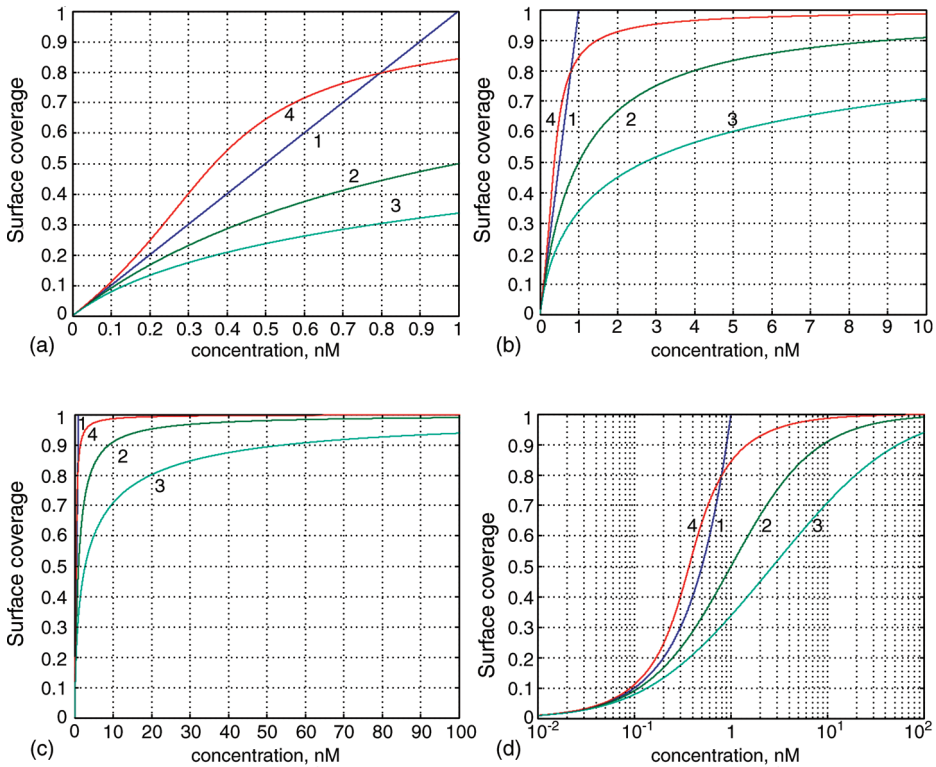


Figure 1.2 Simulated adsorption isotherms according to the models of Henry (curve 1), Langmuir (curve 2) and Frumkin (curves 3 and 4) for attraction ($a = +1$, curve 4) and repulsion

($a = -1$, curve 3) of adsorbed molecules at different concentration scales (a)–(c) and as a semilogarithmic plot (d). Binding constant in all the curves is $1/(1 \text{ nM})$.

such analysis would require information on the coefficient α , which is not always available and in general can be different for different receptors. However, even for unknown but constant values of α , a comparison of slopes of the curves measured at different conditions or for modified receptors provides valuable information on comparison of binding constants.

Binding curves reaching their saturation range are more informative. An analysis of such dependence can provide information not only on the binding constant but also on the homogeneity of the binding sites (or on deviations from the Langmuir model) and on the value of the proportionality factor α corresponding to the saturated value of the signal. The quality of data analysis improves with increasing deviation from linear dependence. However, the maximal concentration that can be used for experimental tests is usually limited (e.g., by solubility or by available amount of the ligand).

Principally, the parameters K and α can be extracted from experimental data by direct nonlinear fitting using Eq. (1.7). Another approach, providing a better

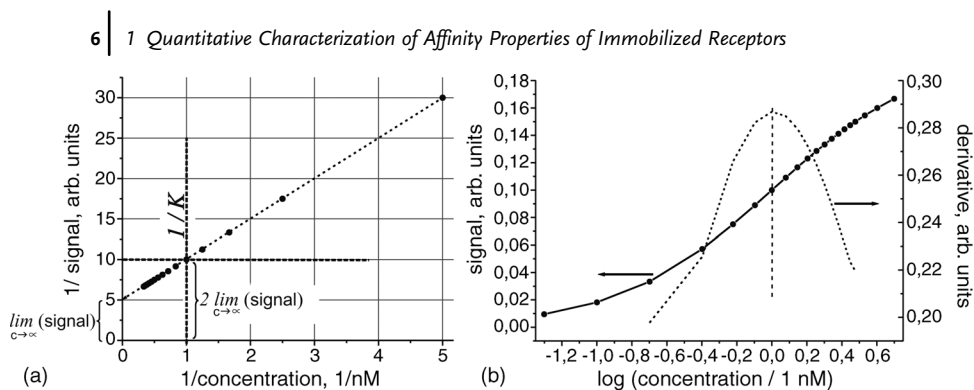


Figure 1.3 Extraction of binding constant by linearization of Langmuir isotherm in double reciprocal plots (a) and by symmetrization in semilogarithmic plots (b). The symmetry point can be found more exactly from the derivative.

visualization of the type of possible deviation from the Langmuir model, is based on linearization of this dependence in a double reciprocal plot (Lineweaver-Burk plot):

$$\frac{1}{S} = \frac{1}{\alpha K c_L} + \frac{1}{\alpha} \quad (1.9)$$

Extrapolation of the line gives information on both parameters of Eq. (1.7) (Figure 1.3a). Although this approach is sometimes considered as a relic of the before-computer era, it has several advantages. For example, it may indicate a systematic deviation from the Langmuir model or heterogeneity of binding sites. In this case, n types of binding sites with different affinity give $n - 1$ breaks of the straight line. Practically it is applicable only for $n = 2$ or 3 and only when the binding constants are very different (about one order or more), and the measurements are performed over the wide concentration range.

Another approach to obtain the binding constant from experimental data measured before saturation is based on data presentation versus a logarithmic concentration scale (Figure 1.2d, curve 2 and Figure 1.3b). In this case the reciprocal binding constant corresponds to the concentration at the symmetry point of the curve. This point also corresponds to the maximal slope or to the point of change of the sign of the curvature. Therefore, for data measured in a small concentration range, where the symmetry point is not clearly visible, it can be also found from analysis of the first or second derivatives (Figure 1.3b).

The obtained binding constant can be then recalculated into the molar standard Gibbs free energy (Gibbs energy) of the binding reaction:

$$\Delta G^0 = -RT \ln K \quad (1.10)$$

The binding constant in Eq. (1.10) characterizes the ratio of activities of corresponding species and is therefore a dimensionless value. By application of usual approximations of activities by concentrations, the concentration values should be

converted into dimensionless ones by normalization of the concentrations to 1 mol l^{-1} .

In practice, the binding constants of immobilized receptors based on equilibrium between volume and surface concentrations are measured under quasi-stationary conditions, as a response of an affinity sensor with this receptor to stepwise increasing ligand concentration. Typically, the measurement is performed 10–40 min after the concentration increase, when the signal “looks” constant. There is no strong criterion on reaching of equilibrium, and this uncertainty may be a reason for a systematic error.

An analysis of binding and selection of an appropriate binding isotherm carry a risk of misinterpretation and require very critical consideration. For example, logarithmic conversion, used for validation of the Freundlich isotherm, leads to strong compression of the variation range and therefore to some data linearization. A break in the double-reciprocal plot of the Langmuir isotherm for two binding sites is observed only for a sufficiently high difference of binding constants of these sites. In addition, data analysis based on a Scatchard plot, which is widely used for affinity experiments in bulk phase, is often misinterpreted [23, 24]. An uncertainty in the definition of quasi-equilibrium was discussed above. In many cases, statements on the selection of adsorption models can be checked by numerical simulations.

1.3 Kinetic Measurements

Binding constants can be also obtained from kinetic measurements. Numerous investigations have demonstrated that the values obtained from kinetic and equilibrium measurements are almost identical. Kinetic measurements of binding constants can be performed much faster than the measurements under quasi-equilibrium conditions. Moreover, for some receptor–ligand pairs the kinetics are so slow that the measurements in quasi-equilibrium conditions are impossible. Another advantage of kinetic measurements is that they provide information not only on the equilibrium binding constant but also on the kinetic constants of binding (k_{ads}) and dissociation (k_{des}).

As is usual for each surface reaction, a process of ligand to receptor binding includes two kinetic steps: diffusion of ligand to the receptor and ligand–receptor binding. A model used for analysis of kinetic measurements is based on the following assumptions: (i) the kinetic limiting step of the whole process is the binding and (ii) the binding can be described by the Langmuir isotherm. It is not a rare case when assumption (i) is not valid. Its validation will be described below.

According to the formal kinetics, the detected adsorption rate $d\theta/dt$ can be described as an algebraic sum of the rates of adsorption and desorption:

$$\frac{d\theta}{dt} = k_{\text{ads}}(1-\theta)c_L - k_{\text{des}}\theta \quad (1.11)$$

or:

$$\frac{d\theta}{dt} = -\theta k_S + k_{\text{ads}}c_L \quad (1.12)$$

and:

$$\frac{dS}{dt} = -Sk_S + k_{\text{ads}}c_L \quad (1.13)$$

where:

$$k_S = k_{\text{ads}}c_L + k_{\text{des}} \quad (1.14)$$

The solution of the differential equation (1.12) is:

$$\theta(t) = [1 - \exp(-k_S t)] \theta_{\text{eq}} \quad (1.15)$$

where θ_{eq} is the surface coverage in equilibrium and can, therefore, be found from Eq. (1.2).

The desorption kinetics observed after replacement of the ligand solution by the solution without ligand can be obtained from the equation:

$$\frac{d\theta}{dt} = -k_{\text{des}}\theta \quad (1.16)$$

The solution with the corresponding starting condition is:

$$\theta(t) = \theta_{\text{eq}} \exp(-k_{\text{des}}t) \quad (1.17)$$

It follows from Eq. (1.12) that k_S is an apparent kinetic constant of adsorption. This value can be obtained from experimental data. It is paradoxical that the desorption constant is one of the components contributing to the adsorption constant in Eq. (1.14).

The dependence of k_S on ligand concentration allows one to obtain values of k_{ads} and k_{des} . The value k_S can be obtained by exponential fitting of adsorption kinetics with Eq. (1.15). Instead of nonlinear fitting, the dependence of dS/dt versus S can be analyzed: a linearity of this dependence verifies the exponential character of the signal kinetics while its slope gives k_S (Figure 1.4). The desorption rate constant k_{des} can also be extracted from fitting of desorption kinetics with Eq. (1.17). This provides a principal way of obtaining kinetic constants of adsorption and desorption and of estimating the binding constant from single experiment. However, desorption kinetics for many receptors are very slow, and k_{ads} cannot be measured directly. In addition, more reliable data can be obtained by repetition of the measurements at different concentrations and by analysis of the concentration dependence of k_S according to Eq. (1.14). Values of k_{ads} and k_{des} obtained from kinetic measurements can be used to calculate the binding constant (K) [Eq. (1.3)]. A deviation of the dependence of k_S versus ligand concentration from a linear dependence may indicate that the process is not limited by the binding step. The quality of this analysis depends strongly on the signal/noise ratio.

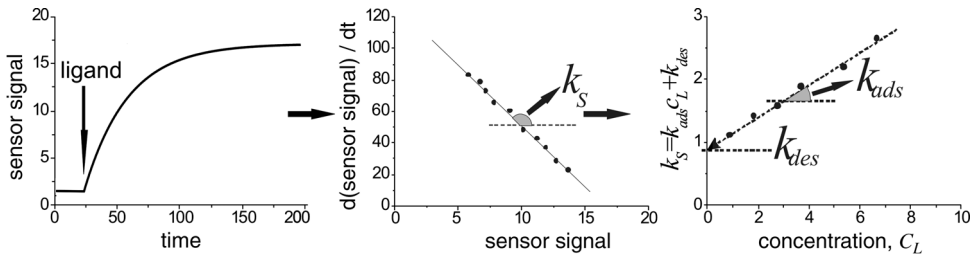


Figure 1.4 Extraction of kinetic constants of adsorption and desorption.

As mentioned above, it is not a rare case that the binding process is limited by diffusion. It is more typical for receptor layers with a three-dimensional matrix. Ignoring this fact and a mechanistic application of the analysis based on the Langmuir model to the diffusion limited processes leads to large errors in the determination of k_{ads} and k_{des} and may lead to considerable errors in binding constants. Therefore it is important to formulate simple criteria to distinguish diffusion and reaction control of the binding process. A deviation of the binding kinetics from mono-exponential dependence is a sign of diffusion limitation; however, it is difficult to provide a reasonable quantitative criterion for this deviation. Moreover, diffusion limited processes can also display a mono-exponential kinetics.

For diffusion controlled processes an adsorption rate depends on the solution viscosity. The viscosity can be modified by addition of sugar, glycerol, or other compounds. The influence of viscosity on binding kinetics should be independent of the chemical nature of the viscosity modifier – a criterion that excludes a chemical influence of the viscosity modifier on the binding process.

Another approach to distinguish diffusion and reaction kinetics is based on variation of thickness of the diffusion boundary layer. It can be performed in devices that are similar to the rotating electrodes widely used in electrochemical experiments. In the case of a reaction limited process, no influence of the rotation rate is expected. For a diffusion limited process, the observed adsorption rate should be increased with increasing rotation rate. The thickness of the hydrodynamic equivalent of the diffusion boundary layer (δ) depends linearly on the square root of reciprocal rotation rate [25]:

$$\delta = 1.61 \cdot D^{\frac{1}{3}} \eta^{\frac{1}{6}} \omega^{-\frac{1}{2}} \quad (1.18)$$

where

D is the diffusion coefficient,

η is the solvent viscosity,

ω is the circular frequency of the electrode rotation.

The diffusion flux is proportional to the reciprocal value of thickness of the diffusion boundary layer. Therefore, for a diffusion controlled process one can expect a linear dependence between the adsorption rate and the square root of the rotation rate. In flow-through cells this approach can be realized by variation of the flow rate.

In cells without through-flow some variation of the thickness of diffusion layer can be obtained by modification of stirring intensity. An application of a rotating receptor surface in immunoassay has been described [26]. Identification of the limiting kinetic step based on modification of viscosity and on investigation of the dependence of binding kinetics on rotation rate of disk electrode has been employed [27]; however, such analysis is time consuming and too difficult for routine applications. A simpler test is an investigation of the influence of flow rate on binding kinetics; a quantitative analysis and experimental validation of this approach has been described [28–31].

1.4 Analysis of Temperature Dependencies

Affinity properties can be further characterized by investigation of the temperature dependencies.

Spontaneous adsorption means that the free energy of the process is negative. Adsorption leads to a decrease in freedom. Taking into account that $\Delta H = \Delta G + T\Delta S$, this means that the enthalpy of adsorption is also negative. Therefore, a binding of a ligand with a receptor leads to heat production. The entropy production can be determined quantitatively by investigation of the temperature dependence of the binding constant. Substitution of $\Delta G = \Delta H - T\Delta S$ in Eq. (1.10) leads to the van't Hoff relationship, which allows one to obtain the molar enthalpy of the binding reaction from the temperature dependence of the binding constant:

$$\ln K = \frac{1}{R} \left(\Delta S^0 - \frac{\Delta H^0}{T} \right) \quad (1.19)$$

Therefore, a slope of the dependence of the logarithm of the equilibrium constant as a function of reciprocal temperature gives the value of standard reaction enthalpy while extrapolation to zero value of the reciprocal temperature gives the value of reaction entropy. A deviation of this dependence was explained by a contribution of a temperature-dependent heat capacity [32]. Assuming a constant difference in heat capacity between free and associated ligand and receptor (ΔC_p) in the temperature range between the temperature of the standard state T_0 (usually 25 °C) and the current temperature T , one can rewrite Eq. (1.19) more exactly:

$$\ln K = \frac{1}{R} \left[\Delta S^0 - \frac{\Delta H^0}{T} + \Delta C_p \left(\frac{T - T_0}{T} - \ln \frac{T}{T_0} \right) \right] \quad (1.20)$$

This provides, in principle, the possibility of determining not only the binding enthalpy and binding entropy but also ΔC_p . However, a numerical simulation demonstrated that the fitting of experimental data is not very sensitive to this parameter, and typical values of experimental errors makes quantitative determination of ΔC_p very uncertain [33].

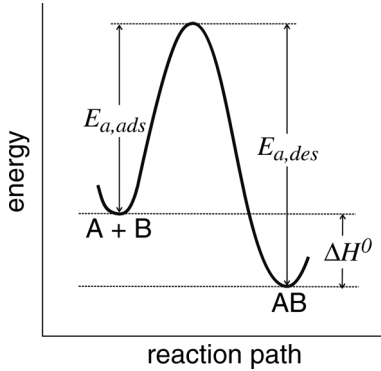


Figure 1.5 Activation energies for binding and dissociation ($E_{a,ads}$ and $E_{a,des}$, respectively) allows us to calculate the molar enthalpy of the binding reaction.

An investigation of temperature dependencies of the kinetic constants of binding (k_{ads}) and dissociation (k_{des}) allows us to obtain activation energies for binding and dissociation, $E_{a,ads}$ and $E_{a,des}$, respectively:

$$k_{ads} \sim \exp(-E_{a,ads}/RT); \quad k_{des} \sim \exp(-E_{a,des}/RT) \quad (1.21)$$

The difference between $E_{a,ads}$ and $E_{a,des}$ can be interpreted as the molar enthalpy of the binding reaction (Figure 1.5):

$$E_{a,ads} - E_{a,des} \approx \Delta H^0 \quad (1.22)$$

The transition state theory developed by Eyring and coauthors [34] provides the principal means of making a more detailed analysis of the reaction profile and to separate the contributions of enthalpy and entropy in the activation process. This theory gives the following equation for the kinetic constant of binding:

$$k_{ads} = \frac{\kappa k_B T}{h} \exp(-\Delta H_{ads}^{\#0}/RT) \exp(-\Delta S_{ads}^{\#0}/R) \quad (1.23)$$

where

- the superscript # indicates that the corresponding thermodynamic potential refers to an activation process,
- k_B is the Boltzmann constant,
- h is the Plank constant,
- κ is a transmission factor, which is considered to be between 0.5 and 1.

Linearization in coordinates $\ln(k/T)$ versus $1/T$ (Eyring plots) can be used to extract values of standard activation enthalpies and entropies; however, a determination of entropy requires an assumption on the value of κ (usually, as $\kappa = 1$).

The risk of misinterpretation of temperature dependencies of equilibrium and kinetic data is very high. Critical analysis of many aspects of this analysis has been performed [33, 35].

1.5

Experimental Techniques

While affinity analysis in the bulk phase can be performed by any analytical technique providing measurements of concentration of free or occupied ligand or receptor molecules, affinity analysis of immobilized receptors requires special methods that provide measurements of surface concentrations. Figure 1.6 gives a short review of the main label-free techniques.

An application of an impedometric approach to study adsorption phenomena came into analytical chemistry from classical electrochemistry. This approach is also known as capacitive detection because, usually, an imaginary component of impedance is used. Obviously, this approach can be applied only for conducting surfaces. In ideal case, the interface should display pure capacitive properties, that is, should block any charge transfer.

The specific electrical capacitance (C) of an interface coated by some dielectric layer with relative dielectric constant ϵ and thickness d is:

$$C = \frac{\epsilon\epsilon_0}{d} \quad (1.24)$$

where ϵ_0 is the dielectric constant of a vacuum. There are two ways to apply capacitive binding detection: to detect changes of either the layer thickness or dielectric constant. The first approach has been used, for example, in spreader-bar systems (the spreader-bar technique is described in Chapter 10) [36, 37]: binding of ligands to receptors leads to an increase of the thickness of the dielectric layer. The second approach has been applied for molecularly imprinted polymers (Chapter 13) [38, 39]:

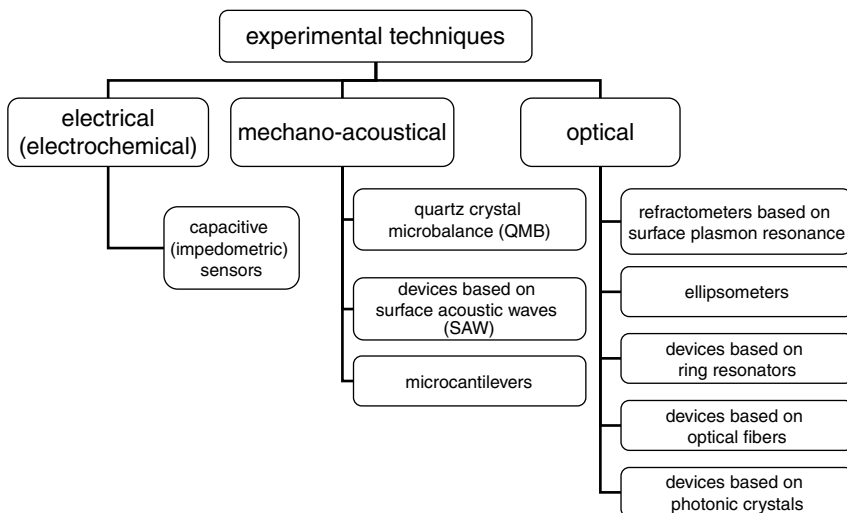


Figure 1.6 Main experimental techniques used for investigation of affinity properties of immobilized receptors.

binding of ligands into the pores of the polymer leads to the replacement of highly polarizable water molecules by lower polarizable ligand molecules and to a corresponding decrease of dielectric constant of the polymer. Linearity between capacitive effect and surface concentration of ligand has been proved for binding of detergents [40]; however, one can expect a strong deviation for large molecules with complex shape [41].

The main types of mechano-acoustic transducers for investigation of ligand–receptor interaction include the quartz crystal microbalance (QCM) [42], devices based on surface acoustic waves (SAWs), and systems based on microcantilevers. The QCM is based on changes of its resonance frequency due to an increase of the mass of the receptor layer caused by binding of ligand. In the gas phase this frequency shift Δf can be described by the Sauerbrey equation [43]:

$$\Delta f = -af_0^2 \Delta m \quad (1.25)$$

where

- f_0 is the resonance frequency of the quartz,
- Δm is the change in mass,
- a is a constant that depends on the quartz material.

The behavior of QCM in viscous phases is more complicated. Commercial QCM devices have been reviewed [44]. According to Eq. (1.25), the sensitivity of a QCM increases as the second power of the resonance frequency; therefore, an increase of this frequency is favorable. However, its increase over 40 MHz requires the formation of crystals that are too thin to provide the required mechanical stability. This limitation can be overcome in SAW-devices, which can operate at hundreds of MHz [45]. Conversely, microcantilevers have a lower Q -factor, lower resonance frequency, and therefore lower sensitivity in dynamic mode. This can probably be compensated for by their high sensitivity in deflection mode. Analytical features of microcantilevers have been reviewed [46].

Sensitive optical methods for label-free detection of ligand interaction with immobilized receptors include surface plasmon resonance (SPR) [47, 48] and several interferometric techniques [49]. The present sensitivity of SPR devices is about 10^{-7} – 10^{-6} refractive index units and is limited by the high temperature dependence of the refractive index of water. Numerous efforts have been made to increase the sensitivity of SPR and to minimize a contribution of bulk water phase.

The methods for analysis of binding properties of immobilized receptors can be applied to obtain complete characterization of the affinity of artificial receptors, including binding constants, adsorption and desorption rate, activation energies for adsorption and desorption, to perform analysis of binding enthalpy and entropy, or even to obtain enthalpy and entropy contributions to activation process during the binding. The main drawback influencing applications of these techniques is the relatively low sensitivity of analytical methods for detection of binding with immobilized receptors. For this reason it may be difficult to obtain reliable results for small ligands.

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