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High-throughput screening: establishing mathematical and physical models for bio-target immobilization

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In high-throughput screening, the immobilization of bio-target shows a significant effect on the target performance, especially the conformation. This, ultimately, has an influence on the screening quality. In present article, a series of mathematical and physical models for target immobilization were established. As shown, in the immobilization process, if the plot of C^*/Q versus C^* is a straight-line, the drugs obtained from in vitro screening are probably applicable to in vivo environment. Otherwise, when the linearity exists between ln Q or Q and ln C^*, particularly between Q and ln C^*, additional competition-experiments of known ligands are necessary. In addition, the immobilization in multilayer form, as well as its impact on screening quality, was also discussed in this article (C^*, the equilibrium concentration of bio-target; Q, the adsorbance).

KEY WORDS: high-throughput screening, target immobilization, theory, mathematical physical models

1. Introduction

Over a long past, the discovery of drugs was mainly based on traditional screening method, resorting to a considerable number of in vitro and in vivo experiments. Such a method is not only a slow, high cost, and low-efficient method, but also results usually in unnecessary waste of human and materials [1–3]. This thus fuels the essential calls for increasing the screening throughput and decreasing the cost. As a result, traditional screening has undergone a profound revolution and evolved currently into high-throughput screening (HTS). In this aspect, as noted, there have been some excellent introductions available [4–6]. Indeed, modern HTS is a multidisciplinary field and involves often biology, biochemistry, molecular biology, analytical chemistry, synthetic chemistry, automation engineering, and computer science, etc. With the control operation,

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HTS is highly intelligentized and automated system, identifying the 'hit' from a large number of diverse chemical structures (against disease targets). Also such a technique needs actually a micro-volume of sample. Now, most pharmaceutical companies are using HTS as the primary engine for the discovery of lead compounds.

With respect to HTS, there is obviously a necessity of mentioning scintillation proximity assay (SPA), the earliest and used likely widest HTS technique at present [7,8]. As evaluated [9,10], in HTS, there are *ca.* 25–50% of cases involving SPA. With this technique in use, the target of interest is immobilized onto a solid support (e.g., SPA beads) that contains a scintillant. When the radiolabeled ligand binds to the target, the radioisotope is brought into close proximity to the support. This thus paves the way for the formation of energy transfer between the emitted beta particles and the scintillant. As a result, the emission of light becomes detectable. Otherwise, the beta particles will dissipate their energy into the aqueous environment and therefore no combination is observed. This is true when the binding is the specific adsorption. To decrease the nonspecific adsorption, as well as to cut down the interaction from the support, SPA beads are often with the surface of poly-hydroxyl groups [11]. So far, this technique has been widely applied not only in common screening process, but also in cellular adhesion binding, protein–peptide interaction, protein–DNA interactions, and biochemistry assays, etc [12–14]. Nowadays, other assay techniques for HTS such as affinity chromatography (AC) [15], fluorescence-linked immunosorbent assay (FLISA) [16], alpha screening [17], nuclear magnetic resonance (NMR) [18], surface plasmon resonance (SPR) [19], and various advanced fluorescent techniques [20,21] are also available.

As noted, much investigation has revealed that unlike the coupling of small molecules, the immobilization of bio-target is actually considerably complicated, which involves often various interactions including electrostatic, hydrophobic, and hydrogen-bonding, etc. [22–24]. As a result, the immobilization in SPA screening shows always a significant effect on target performance, especially the conformation. This is actually also evidenced in other screening methods, such as AC, FLISA, and alpha screening, etc. [25,26]. As well known in HTS, the difference of target conformation between in vitro and in vivo can lead to an invalid screening, although the obtained candidates are effective in vitro. Unfortunately, due to the complexity of practical process, almost no discriminance or theoretical guide for these cases is currently available. More works especially the theoretical works, no doubt, is necessary. As a supplement, a series of mathematical and physical models for bio-target immobilization were presented here. To help learning the nature behavior of macromolecular immobilization, related theories were developed. The purpose is to reveal some important information, especially the nature information, involved by the process of target immobilization.

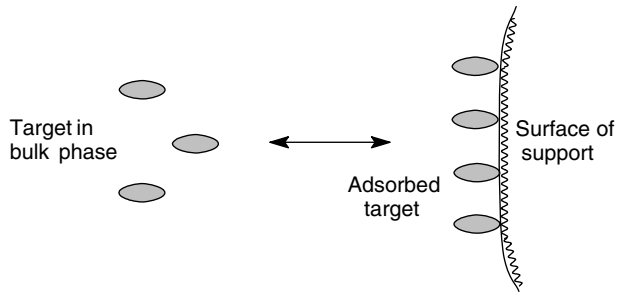

Figure 1. Ideal model of target immobilization.

2. Establishment of ideal model

Models are the basis of developing related theories. To conveniently elucidate, figure 1 presents a physical model that is being used widely in HTS for the immobilization of target. In the bulk phase, target molecules, due to the interaction from the support, are absorbed onto the surface, while over the surface, aroused by heat movement, the absorbed target trends to escape from the surface. As a result, the total rate of adsorption is determined by both adverse factors [27, 28]:

$$\begin{aligned} v_t &= v_{\mathrm{a}} - v_{\mathrm{d}} \\ &= k_a S(1-\theta)C - k_{\mathrm{d}} S\theta. \end{aligned} \tag{1}$$

Here v_{a} and v_{d} are, respectively, the rates of adsorption and desorption, k_{a} and k_{d}, the corresponding rate constants, C, the concentration of target in the bulk phase, θ, the coverage degree of adsorbate over the surface, and S, the total combinable surface-area of the support. In the beginning, v_{a} shows a maximal value (due to the maximal C). Subsequently, v_{a} decreases with declining C, while v_{d} increases with enhancing θ. With time running on, this, ultimately, leads to an equilibrium between adsorption and desorption. Clearly, in the equilibrium, no macroscopical adsorption is observed:

$$\begin{aligned} v_t &= v_{\mathrm{a}} - v_{\mathrm{d}} = 0 \\ &= k_{\mathrm{a}} S(1-\theta)C^* - k_{\mathrm{d}} S\theta. \end{aligned} \tag{2}$$

Here the symbol '*' characterizes the equilibrium, distinguishing from the non-equilibrium point. Now, the solution to equation (2) will give:

$$\theta = \frac{KC^*}{1 + KC^*}. \tag{3}$$

Here K is the thermodynamic constant (coming from $K = k_a/k_d$). The substitution of $\theta = Q/Q_m$ to equation (3) shows:

$$\frac{Q}{Q_m} = \frac{KC^*}{1 + KC^*}. \tag{4}$$

For application, equation (4) can be sought into such form:

$$\frac{C^*}{Q} = \frac{1}{KQ_m} + \frac{1}{Q_m}C^*. \tag{5}$$

Here Q and Q_m are the actual and maximal adsorbance. Clearly, according to equation (5), if the plot of C^*/Q versus C^* is a straight-line, no intermolecular interaction is normally expected to involve in the immobilization process. By the slope and intercept, the constant K, characterizing the affinity of target to support, can be obtained. In literature, the applicability of equation (5) to some immobilization cases is clear [29–31]. However, it is necessary to point out that there are more factors requiring consideration in actual HTS process. As shown in figure 2, if the binding site is at the end-point of bio-target, the effect of end-point immobilization on the whole conformation of biomacromolecules is relatively less. As a result, the drugs obtained from in vitro screening are still probably applicable to in vivo environment. Otherwise, when the middle of biomacromolecules becomes the binding site, the effect of immobilization is actually remarkable, which probably results in a severe torsion or deformation of bio-target conformation. This, eventually, can lead to an invalid screening. Thus, for the case involving the binding in the middle, additional competition-experiments of known ligands are necessary.

3. Establishment of interacted model

Relating to small molecules, most immobilizations of bio-targets are much more complicated and can involve various interactions among themselves, or that with support (figure 3). This, to some extent, is associated with the structure

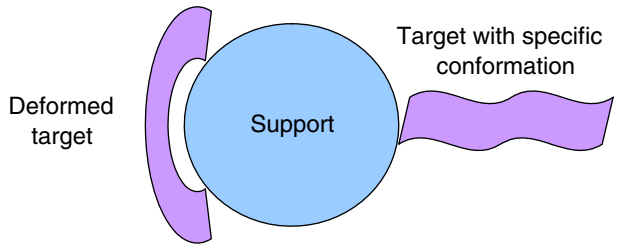

Figure 2. Physical profile for binding mode in target immobilization.

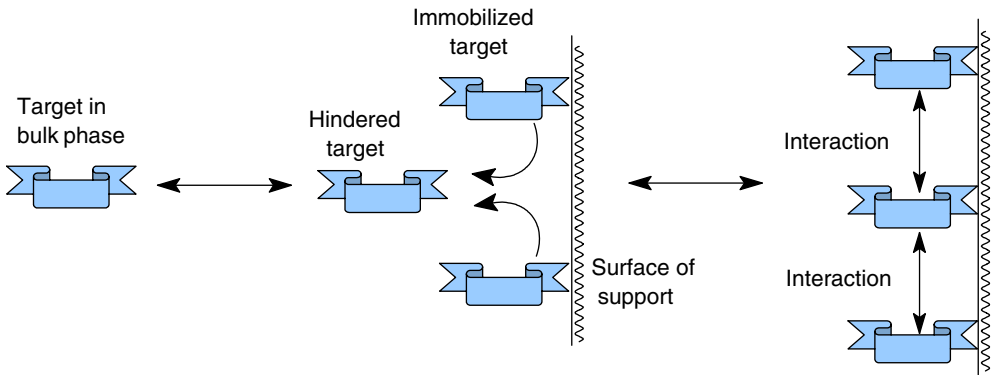

Figure 3. Physical profile for the adsorption with intermolecular interactions intervening.

of bio-target [22,23]. Bio-target, such as protein, consists of a series of amino-acid units, and parts of them, with side chain of carboxyl or amino groups. In the adsorption process, this structure may introduce a large number of intermolecular interactions such as electrostatic, hydrophobic, hydrogen-bonding, and so on. Moreover, with the adsorption running on, more biomacromolecules are adsorbed. The earlier adsorbed macromolecules will inevitably hinder sterically the latter adsorption. As a result, in progress, the adsorption heat, as well as the activation energy, is not a fixed value but related to coverage degree. Hence, in most cases, the adsorptive or desorptive rate is actually a function of the coverage degree [32,33]:

$$\begin{aligned} v_{\mathrm{a}} &= k_{\mathrm{a}}(\theta) S(1-\theta) C \\ &= a \exp\left[-\frac{E_{\mathrm{a}}(\theta)}{RT}\right] \times S(1-\theta) C, \\ v_{\mathrm{d}} &= k_{\mathrm{d}}(\theta) S\theta \\ &= b \exp\left[-\frac{E_{\mathrm{d}}(\theta)}{RT}\right] \times S\theta. \end{aligned} \tag{6}$$

Here the new items are coming from the dispersion of Ahrrenius equation. In the equilibrium point, there is:

$$a \exp\left[-\frac{E_{\mathrm{a}}(\theta)}{RT}\right] \times S(1-\theta) C^* = b \exp\left[-\frac{E_{\mathrm{d}}(\theta)}{RT}\right] \times S\theta \tag{7a}$$

or

$$\begin{aligned} \frac{\theta}{1-\theta} &= \frac{a}{b} \exp\left[-\frac{E_{\mathrm{a}}(\theta) - E_{\mathrm{d}}(\theta)}{RT}\right] C^* \\ &= A \exp\left[\frac{q_{\mathrm{m}}(\theta)}{RT}\right] C^*. \end{aligned} \tag{7b}$$

Here A is a constant (obtained from $A = a/b$), and $q_{\mathrm{m}}(\theta)$, the adsorption heat. In most occasions, as summarized, the change of $q_{\mathrm{m}}(\theta)$versus θ can be classified into logarithmic and linear kinds [28, 32]:

$$q_{\mathrm{m}}(\theta) = q_{\mathrm{m}}^{0} - \alpha \ln \theta, \tag{8a}$$

$$q_{\mathrm{m}}(\theta) = q_{\mathrm{m}}^{0} - \beta\theta. \tag{8b}$$

Here both $\alpha$and β are constants, and q_{m}^{0}, the original adsorption-heat. Now, the substitution of equation (8a) to (7b) gives:

$$\theta^{\alpha/RT}\left(\frac{\theta}{1-\theta}\right) = A\exp\left(\frac{q_{m}^{0}}{RT}\right)C^{*}. \tag{9}$$

On the left of equation (9), the change of θ in exponential form is much larger than that in fraction form. Thus, relating to the exponential expression, the effect of fraction moiety is actually minor, especially in a middle coverage-degree. As a reasonable approximation, equation (9) can be further sought into such a form:

$$\begin{aligned}\theta &= \left[A\exp\left(\frac{q_{\mathrm{m}}^{0}}{RT}\right)C^{*}\right]^{RT/\alpha} \\ &= K\,(C^{*})^{1/n}.\end{aligned} \tag{10a}$$

or

$$\frac{Q}{Q_{\mathrm{m}}} = K\left(C^{*}\right)^{1/n}. \tag{10b}$$

Here K and n are constants under a specific condition (obtained, respectively, from $K = \left[A\exp(q_{\mathrm{m}}^{0}/RT)\right]^{RT/\alpha}$ and $n = \alpha/RT$). For convenient use, equation (10) can be rewritten into:

$$\ln Q = \frac{1}{n}\ln C^{*} + \ln K Q_{\mathrm{m}}. \tag{11}$$

Apparently, equation (11) is exactly the basis expression of Freundlich model. According to such a model, if the plot of $\ln Q$ versus $\ln C$ appears to be a straight-line, the intermolecular interactions are normally expected to involve in the immobilization process. In literature, the efficiency of equation (11) in various immobilization processes has been well confirmed, such as antibody or antigen, protein, and enzyme, etc. [34, 35].

Now, similar to above treatment, replacing of equation (8b) into (7b) will show:

$$\begin{aligned}\frac{\theta}{1-\theta} &= A\exp\left(\frac{q_{\mathrm{m}}^{0}}{RT}\right)\exp\left(-\frac{\beta\theta}{RT}\right)C^{*} \\ &= \exp\left(-\frac{\beta\theta}{RT}\right)C^{*}.\end{aligned} \tag{12a}$$

Here f is a constant, coming from the unification of various constants. For treat, one can seek equation (12a) into the logarithm form:

$$\ln C^* = \frac{\beta}{RT}\theta - \ln f + \ln\left(\frac{\theta}{1-\theta}\right). \tag{12b}$$

In the middle coverage degree, the value of $\theta/(1-\theta)$ is approximated to 1. Clearly, this will lead to:

$$\ln C^* = \frac{\beta}{RT}\theta - \ln f \tag{13a}$$

or

$$\theta = \frac{RT}{\beta}\ln fC^*. \tag{13b}$$

Now, the substitution of $\theta = \frac{Q}{Q_m}$ to equation (13b) will give:

$$Q = K\ln C^* + K\ln f. \tag{14}$$

Here, K is also a constant. Obviously, like equation (11), equation (14) describes also the immobilization behavior in the presence of intermolecular interactions. In our previous work [34], pepsin was immobilized onto the aldehyde-modified PMMA microspheres. The result shows that equation (14) can summarize efficiently this process. Some similar observations are also shown in other works [36,37]. As noted, the provenance of equation (14) is based on the linear change of adsorption heat versus coverage degree, while equation (11) is derived from the logarithmic decrease. Hence, in comparison to equation (11), equation (14) describes actually a stronger interaction system. In HTS, as already mentioned, the validity of drugs-obtained from in vitro screening depends directly on the screening environment. As already mentioned, the presence of intermolecular interactions has an effect on the target conformation. Thus, this likely brings a negative impact into the final screening-quality. As a result, for the immobilizations obeying equations (11)–(14), particularly equation (14), there is an obvious necessity of using more competition-experiments to check up the reliability of these drugs.

4. Establishment of multilayer model

As mentioned, the immobilization of bio-target can involve a large number of intermolecular interactions. In progress, these interactions likely induce multilayer form of adsorption into the system [27,33]. As shown in figure 4, a series of adsorptions in succession, due to the presence of electrostatic, hydrophobic or hydrogen-bonding interaction, are established over the surface of original molecules adsorbed. Clearly, the interaction subjected in the first layer is

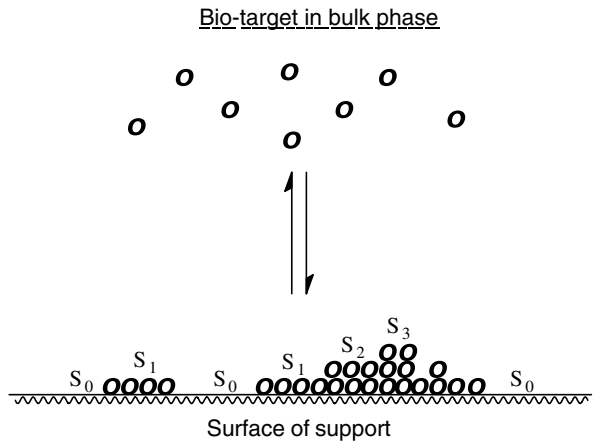

Figure 4. Physical profile for target immobilization in multilayer form.

different from those in other layers. Also the formation of succedent adsorptions does not require the completion of anterior adsorption. To conveniently treat, we assume that S_0, S_1, S_2, ..., S_i, ..., are, respectively, the surface area of covering 0, 1, 2, ..., i, ..., layers of target. Apparently, in the equilibrium point, there is such a relationship existing in the first layer:

$$a_1 C S_0 = b_1 S_1 e^{-q_1/RT}. \tag{15}$$

This available is according to the dispersion of Langmuir model (with Ahrrenius equation). Here a_1 and b_1 are constants, coming from the pre-exponential factors, and q_1, the adsorption heat of first layer. Similarly, for two layers of equilibrium, there is

$$a_1 C S_0 + a_2 C S_1 = b_1 S_1 e^{-q_1/RT} + b_2 S_2 e^{-q_2/RT}. \tag{16}$$

Now, substituting of equation (15) into (16) will show:

$$a_2 C S_1 = b_2 S_2 e^{-q_2/RT}. \tag{17}$$

Analogously, for the adsorption in more layers, there will be

$$\begin{aligned} a_3 C S_2 &= b_3 S_3 e^{-q_3/RT}, \\ &\cdots\cdots\cdots\cdots\cdots \\ a_i C S_{i-1} &= b_i S_i e^{-q_i/RT}. \\ &\cdots\cdots\cdots\cdots\cdots \end{aligned} \tag{18}$$

Clearly, the total surface area for molecular adsorption is

$$S_t = \sum_i S_i, \tag{19}$$

and the adsorbance is

$$Q = Q_0 \sum_i i S_i. \tag{20}$$

Here Q_0 is the specific adsorbance with monolayer adsorption in a unit of surface. Now, the correlation of equations (20) with (19) will give:

$$\frac{Q}{S_t Q_0} = \frac{Q}{Q_\mathrm{m}} = \frac{\sum_i i S_i}{\sum_i S_i}. \tag{21}$$

Here Q_m is the adsorbance of saturated adsorption in monolayer. As already mentioned, except for the first layer, the interaction subjected in other layers is basically same. Thus, as a reasonable approximation, one can expect such a relation among other layers:

$$\begin{aligned} &q_2 = q_3 = \cdots = q_i = \cdots = q_L, \\ &\frac{b_2}{a_2} = \frac{b_3}{a_3} = \cdots = \frac{b_i}{a_i} = \cdots = g. \end{aligned} \tag{22}$$

Here both $q_L$and g are constants under specific condition. When treating equation (21), there is obviously a necessity of correlating S_1, S_2, …, S_i, …, with S_0. Now, assuming that $(C/g)e^{q_L/RT} = x$ and $(a_1/b_1)Ce^{q_1/RT} = y$, then the substitutions of x and y to equations (15), (17), and (18) will show:

$$\begin{aligned} &S_1 = yS_0, \\ &S_2 = xS_1 = xyS_0, \\ &S_3 = x^2 yS_0, \\ &\cdots\cdots\cdots\cdots \\ &S_i = x^{i-1} yS_0. \\ &\cdots\cdots\cdots\cdots \end{aligned} \tag{23}$$

To conveniently solve, assuming again that $\dfrac{y}{x} = \dfrac{a_1 g}{b_1} e^{(q_1 - q_L)RT} = A_0$, so that S_i can be sought into such form:

$$S_i = A_0 x^i S_0. \tag{24}$$

Substituting it into equation (21) will show:

$$\frac{Q}{Q_\mathrm{m}} = \frac{A_0 S_0 \sum_{i=1} i x^i}{S_0[1 + A_0 \sum_{i=1} x^i]}. \tag{25}$$

For the denominator, one can easily obtain:

$$\sum_{i=1} x^i = \frac{x}{1-x}. \tag{26}$$

Similarly, the treatment of numerator will show:

$$\sum_{i=1} ix^i = \frac{x\partial\left(\sum_i x^i\right)}{\partial x} = \frac{x}{(1-x)^2}. \tag{27}$$

Now, the substitution of equations (26) and (27) to (25) gives:

$$\frac{Q}{Q_{\mathrm{m}}} = \frac{A_0 x}{(1-x)(1-x+A_0 x)}. \tag{28}$$

Since the adsorption is in a multi-layer form, there, in theory, may present the infinite adsorbance. Hence, in order to reach $Q \to \infty$, the numerator must remain as an infinitesimal value. As a result, the choice for x is left by 1 or $1/(1-A_0)$. As well known in often case, the interaction subjected in the first layer is generally stronger than those in other layers. This leads to that A_0 becomes bigger than 1. Thus, such a value $1/(1-A_0)$ for x is almost meaningless. Clearly, in the point that $x = 1$, there presents:

$$(C_0/g)e^{q_L/RT} = 1. \tag{29}$$

Here C_0 is the saturated concentration, corresponding to the saturate adsorption. Now, the correlation of $x = (C/g)e^{q_L/RT}$ with equation (29) will give:

$$x = \frac{C}{C_0}. \tag{30}$$

The substitution of equation (30) to (28) can show:

$$\frac{Q}{Q_{\mathrm{m}}} = \frac{A_0 C}{(C_0 - C)[1 + (A_0 - 1)C/C_0]}. \tag{31}$$

For convenient application, equation (31) can be rewritten into:

$$\frac{C}{Q(C_0 - C)} = \frac{1}{Q_{\mathrm{m}} A_0} + \left(\frac{A_0 - 1}{Q_{\mathrm{m}} A_0}\right)\frac{C}{C_0}. \tag{32}$$

Apparently, in HTS, if the plot of $C/[Q(C_0 - C)]$ versus C is a straight-line, the immobilization is normally expected to be in a multi-layer form. As mentioned, the final quality of screening drugs is directly dependent on in vitro screening-environment. In multi-layer immobilization, the formation of succedent adsorptions is mainly based on intermolecular interactions. Clearly, if the effect of these interactions on target performance, particularly on the conformation, is not too much, the drugs obtained are probably applicable to in vivo environment. Otherwise, the additional competition-experiments of known ligands are necessary. Recently, more and more investigations indicate that the drugs obtained from multilayer immobilization are applicable to in vivo environment [10, 28, 32].

5. Final remarks

Based on the multidisciplinary background, HTS is a considerably complicated process. As the earliest and used likely widest HTS technique at present, the applicability and reliability of SPA to various assays has been well confirmed. With such a technique in use, the bio-target is immobilized onto a solid support. As known, this immobilization can show significant effect on the target performance, particularly on the conformation, and therefore has effect on the quality of drug screening. These are also apparent in other screening techniques, such as AC, FLISA, and alpha screening, etc. Unfortunately, due to the complexity of practical process, almost no discriminance or theoretical guide for these cases is currently available. In this article, a series of mathematical and physical modes was established. As shown, in the immobilization process, if the plot of C^*/Q versus C^* is a straight-line, the drugs obtained from in vitro screening is probably applicable to in vivo environment. Otherwise, when the linearity exists between ln Q or Q and ln C^*, particularly between Q and ln C^*, additional competition-experiments of known ligands are necessary (C^*, the equilibrium concentration of bio-target; Q, the adsorbance). It is also necessary to point out that some opinions in the present article are likely somewhat tentative; further work is necessary and is currently underway. Other aspects that, have not, as yet, been addressed here, are also expected to be significant.

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