LIGAND-RECEPTOR INTERACTIONS
Including their Role in Immunology
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Energetics and Kinetics of Specific Ligand-Receptor (Including Antigen-Antibody) Interactions

Specific ligand-bearing peptides with pre-existing (i.e., innate) anti-microbial specificities exist in the entire biological realm, from humans and other animals, plants, molds and bacteria. One example of these is lysozyme, discovered in 1920 by Sir Alexander Fleming. In the same, innate, category as these peptides are the omni-present lectins, which are proteins or glycoproteins (with specificities against carbohydrates), such as human blood cell antigens, with Concanavalin A as a major example.

Defense macromolecules with specific ligands of a more recent vintage (from an evolutionary point of view) are antibodies (Ab) whose specificities are adaptive (i.e., acquired during the host's life); these are created after a first encounter with the antigens (Ag) of given invading foreign infectious agents. Ab's are blood serum proteins with both adaptive and innate properties. The specific ligands (paratopes) of Ab's comprise about six amino acids and are concave and often relatively hydrophobic; they can fit into the complementary shaped combining sites or receptors (epitopes) of Ag's, which are convex and hydrophilic.

The forces between paratopes and epitopes are various combinations of the three non-covalent Lifshitz-van der Waals (LW), Lewis acid-base (AB) and electrostatic (EL) forces. The optimal specificity of the interaction between an Ag and its specific Ab, is reached by achieving the best fit and therefore the shortest distance, between the contactable surfaces of the protruding epitope and the hollow paratope, resulting in the strongest possible binding energies. The energetics and kinetics of Ag-Ab binding are similar to those of the physical adsorption between, e.g., macromolecules and solid surfaces, when immersed in water.

The binding energy is proportional to the natural logarithm of the equilibrium binding constant (i.e., ln $K_a$), where:

$$K_a = \frac{k_a}{k_d}$$

and where $k_a$ and $k_d$ are, respectively, the kinetic association (a) and dissociation (d) rate constants. It has been shown experimentally that the great variability between the binding energies among different specific ligand-receptor systems is mainly function of the kinetic dissociation rate constant, $k_d$, i.e., of the ease or difficulty, or more precisely the speed, with which the ligand and receptor will separate from one another, immediately after having been bound together. This is also the case in, for instance, protein-silica adsorption systems, where some of the pertinent data were based on contact angle measurements.
LIGAND-RECEPTOR INTERACTIONS

SPECIFIC

INNATE: SPECIFIC BIOPOLYMERS ONE IS BORN WITH (≈ 1,000 SPECIFICITIES)

ADAPTIVE: SPECIFIC BIOPOLYMERS ONE FORMS THROUGH ENCOUNTER AFTER BIRTH (≈ 1,000,000 SPECIFICITIES)

INNATE: SMALL CATIONIC ANTIL MICROBIAL PEPTIDES (E.G.: LYSOZYME)

PLANT LECTINS (E.G.: CONCANAVALIN-A, PHYTOHEMAGGLUTININS)

ADAPTIVE: ANTIBODIES (I.E.: IMMUNOGLOBULINS) (MADE BY B-CELLS)
Furthermore, there are many innate helper proteins, serving, e.g., as messengers to activate phagocytic and other killer cells.

Examples:

Complement factors, other cytokines

**Cells**

**Phagocytic cells:**

1st Line of defence

- Polymorphonuclear cells, especially neutrophils

2nd Line of defence

- Monocytes, which become macrophages

**Killer cells**

- T cells
- NK (natural killer) cells

Lymphocytes
ANTIBODIES: ADAPTIVE!

ANTIBODIES ARE IMMUNOGLOBULINS; IN HUMANS:

<table>
<thead>
<tr>
<th>SERUM %</th>
<th>Ig M, Ig G, Ig A, Ig E, Ig D</th>
<th>10 2 2 2 2 VALENCIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Ig M; Mw= 900,000; PRIMARY RESPONSE</td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>Ig G; u= 150,000; SECONDARY RESP</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>Ig A; u= 155,000; SECRETORY AB'S</td>
<td></td>
</tr>
<tr>
<td>0.0001</td>
<td>Ig E; u= 185,000; ALLERGIES; ANTI-PARASITE</td>
<td></td>
</tr>
<tr>
<td>0.003</td>
<td>Ig D; u= 187,000; NEWBORNS; PREAM PRIMARY</td>
<td></td>
</tr>
</tbody>
</table>

AFTER FIRST ENCOUNTER WITH NEW AG, AB'S TAKE ~ ONE WEEK TO DEVELOP; AFTER SECOND ENCOUNTER: MUCH FASTER. TOTAL # OF SPECIFICITIES: ~ 1,000,000

Ig G IS THE ONLY Ig THAT PASSES THE PLACENTA; HALF-LIFE OF Ig G ~ 3 WEEKS
IgG
IgM
ANTIGENS

BIOPOLYMERS:  (Most frequently):

PROTEINS
GLYCOPROTEINS

ALSO: GLUCIDES (e.g.: Blood group sugars)

DNA (Only pathologically)

SMALLER MOLECULES:

HAPTENS (e.g.: DNP + 1 amino acid, i.e.: THE ANTIGEN HERE IS JUST THE EPITOPE)

SYNTHETIC POLYMERS:  (Very rarely):

POLYVINYL PYRROLIDONE (PVP) IS WATER-SOLUBLE AND THE ONLY ACTIVE ANTIGEN OF THE GROUP (INACTIVE)

HYDROPHOBIC: Polybutadiene, polystyrene, silicones

HYDROPHILIC: PEO, polyvinyl alcohol, polyvinyltoluene, polyacrylic acid, polystyrene-sulphonic acid
EPITOPES

Ag
Ag-Ab INTERACTIONS - 1

occur via the:

antigen-specific site, or:

**EPI TOPE**

plus the antibody-active site, or:

**PARAT OPE**

the epitope is convex and hydrophilic;

the paratope is concave and usually at least partly hydrophobic.

their bond is strongest at best fit

because then XDLVO attraction is strongest at shortest distance
Ag-Ab INTERACTION ENERGIES.

THE NON-COVALENT INTERACTIONS: LW, AB AND EL INTERACTION ENERGIES AS A FUNCTION OF DISTANCE USING THE EXTENDED DLVO THEORY, BY INCLUDING THE LEWIS ACID-BASE APPROACH (AB ENERGIES) REPRESENTING 90% OF THE INTERACTION ENERGIES OCCURRING IN WATER!

LAW OF MASS ACTION:

\[ \text{Ag} + n.\text{Ab} \leftrightarrow \text{Ag}.\text{Ab}_n \]  \[ 1 \]

THEN:

\[ K_a = \frac{[\text{Ag}.\text{Ab}_n]}{[\text{Ag}].[\text{Ab}]^n} \]  \[ 2 \]

FURTHERMORE:

\[ \frac{\Delta G_{i\text{w}2}^E \times S_c}{kT} = \frac{(kT)}{kT} \]  \[ 3 \]

AND:

\[ \Delta G_{i\text{w}2}^{(\text{in } kT)} = -\ln(K_a \times 55.6) \]  \[ 4 \]
A\textsubscript{g}-Ab INTERACTIONS - 3

ENERGETICS & POLYCLONAL VS. MONOCLONAL ANTIBODIES

Up to the end of the 20\textsuperscript{th} century one only had polyclonal Ab\textsuperscript{S} & plurivalent Ag\textsuperscript{S}, resulting in Ag-Ab complexes of mixed Ag/Ab ratios leading to a non-solvable:

\[ K_a = \frac{[Ag\textsubscript{n}-Ab\textsubscript{m}]}{[Ag]^n \cdot [Ab]^m} \tag{2-bis} \]

with unknown \( n \) and \( m \) values and no meaningful stoichiometry nor a useable Eq. 4!

However, since G. Köhler & C. Milstein (1975), Nature 256, 495, monoclonal Ab\textsuperscript{S} became possible
Figure 7.4 Composition of three typical Ag–Ab complexes at different Ag/Ab ratios.
Typical graph of the various amounts of Ag–Ab precipitate formed as a function of excess, optimal Ag/Ab ratio and Ag excess.
### TABLE VI-1  Energies of interaction ($\Delta G^{LW}_{\ell}$) and forces of interaction of ($F^{LW}_{\ell}$) of unretarded Lifshitz–van der Waals interaction, for a number of configurations, as a function of distance, $\ell$

<table>
<thead>
<tr>
<th>Configuration</th>
<th>$\Delta G^{LW}_{\ell}$</th>
<th>$F^{LW}_{\ell}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(semi-infinite flat parallel slabs)</td>
<td>$-\frac{A}{12\pi\ell^2}$</td>
<td>$\frac{A}{12\pi\ell^3}$</td>
</tr>
<tr>
<td>(sphere of radius R and semi-infinite flat slab: also valid for two crossed cylinders at 90°)</td>
<td>$-\frac{AR}{6\ell}$</td>
<td>$\frac{AR}{6\ell^2}$</td>
</tr>
<tr>
<td>(two spheres of radius R)</td>
<td>$-\frac{AR}{12\ell}$</td>
<td>$\frac{AR}{12\ell^2}$</td>
</tr>
</tbody>
</table>

### TABLE VI-2  Energies of interaction ($\Delta G^{AB}_{\ell}$) and forces of interactions ($F^{AB}_{\ell}$) of polar [electron-acceptor–electron-donor, or Lewis acid-base (AB)] interactions, for a number of configurations, as a function of distance, $\ell^*$

<table>
<thead>
<tr>
<th>Configuration</th>
<th>$\Delta G^{AB}_{\ell}$</th>
<th>$F^{AB}_{\ell}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(flat parallel plates)</td>
<td>$\Delta G^{AB}_{\ell}$ exp[($\ell_o - \ell$)/$\lambda$]</td>
<td>$F^{AB}_{\ell}$ exp[($\ell_o - \ell$)/$\lambda$]</td>
</tr>
<tr>
<td>(sphere of radius R and flat plate: also valid for two crossed cylinders at 90°)</td>
<td>$2\pi R \lambda \Delta G^{AB}_{\ell}$ exp[($\ell_o - \ell$)/$\lambda$]</td>
<td>$-2\pi R \lambda \Delta G^{AB}_{\ell}$ exp[($\ell_o - \ell$)/$\lambda$]</td>
</tr>
<tr>
<td>(two spheres of radius R)</td>
<td>$\pi R \lambda \Delta G^{AB}_{\ell}$ exp[($\ell_o - \ell$)/$\lambda$]</td>
<td>$-\pi R \lambda \Delta G^{AB}_{\ell}$ exp[($\ell_o - \ell$)/$\lambda$]</td>
</tr>
</tbody>
</table>

*$\Delta G^{AB}_{\ell}$ is obtained from eqs. [III-6], [III-16], or [III-17], and $F^{AB}_{\ell} = (1/\lambda) \Delta G^{AB}_{\ell}$, or $F^{AB}_{\ell}$ is measured experimentally. The superscript * indicates that $\Delta G^{\prime}$ or $F^{\prime}$ were obtained at the plane parallel plate configuration, at the minima equilibrium distance $\ell_o$.

### TABLE VI-3  Energies of interaction ($G^{FL}_{\ell}$) and forces of interactions ($F^{FL}_{\ell}$) of electrostatic interactions for a number of configurations, as a function of distance, $\ell^*$, for relatively weak interactions, i.e., for $\xi < 25 \text{ mV}$

<table>
<thead>
<tr>
<th>Configuration</th>
<th>$\Delta G^{FL}_{\ell}$</th>
<th>$F^{FL}_{\ell}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(flat parallel plates)</td>
<td>$\frac{1}{\kappa} \cdot 64n k T \gamma_o^2 \exp(-\kappa \ell)^b$</td>
<td>$-64n k T \gamma_o^2 \exp(-\kappa \ell)^b$</td>
</tr>
<tr>
<td>(sphere of radius R and flat plate: also valid for two crossed cylinders at 90°)</td>
<td>$\varepsilon R \psi_o^2 \ln[1 + \exp(-\kappa \ell)]$</td>
<td>$-\varepsilon \kappa R \psi_o^2 \ln[1 + \exp(-\kappa \ell)]$</td>
</tr>
<tr>
<td>(two spheres of radius R)</td>
<td>$0.5\varepsilon R \psi_o^2 \ln[1 + \exp(-\kappa \ell)]$</td>
<td>$-0.5\varepsilon \kappa R \psi_o^2 \ln[1 + \exp(-\kappa \ell)$</td>
</tr>
</tbody>
</table>

*For explanation of the symbols used, see Chapter IV, eqs. [IV-2–IV-6].

$^b\gamma_o = [\exp(\psi_o/2kT) - 1]/[\exp(\psi_o/2kT) + 1]$ (eq. [IV-4]).
Table 2. Free energies of interaction as a function of distance, \( \ell \) (\( \Delta G \)) for the interactions between two equal spheres of radius, \( R \) and between one such sphere and a flat plate (cf. van Oss [1994, pp. 75 88])

<table>
<thead>
<tr>
<th>( \Delta G )</th>
<th>( \Delta G^{(0)} )</th>
<th>( \Delta G^{(f)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-AR/12\ell^a)</td>
<td>( \pi RL \Delta G_{\ell}^{(0)} \exp \left[ \left( \ell_0 - \ell \right) / \lambda \right] )</td>
<td>( 0.5 \epsilon R \gamma_{0}^{2} \ell n[1 + \exp(-\kappa \ell)] )</td>
</tr>
<tr>
<td>(-AR/6\ell^b)</td>
<td>( 2\pi RL \Delta G_{\ell}^{(s)} \exp \left[ \left( \ell_0 - \ell \right) / \lambda \right] )</td>
<td>( \epsilon R \gamma_{0}^{2} \ell n[1 + \exp(-\kappa \ell)] )</td>
</tr>
</tbody>
</table>

**Extended DLVO Theory:**

**Influence of \( R \)**
\[ K_a = \frac{k_a}{k_d} \quad [5] \]

\[ K_a \text{ in } L/M \quad [6] \]

\[ k_a \text{ in } L/(M \text{sec}) \quad [7] \]

\[ k_d \text{ in } 1/\text{sec} \quad [8] \]

\[ K_{ed} = \frac{k_d}{k_a} = \frac{1}{K_a} \quad [9] \]

- \( K_{ed} \) and \( k_d \) CANNOT BE MEASURED
  DIRECTLY DUE TO HYSTERESIS

- NEVER USE \( K_d \)!

- DETERMINE \( k_d \) VIA: \( k_d = \frac{k_a}{K_a} \quad [10] \)
KINETICS - 2

$K_a \& k_a$ BEST DETERMINED AT TIME $\to 0$

$K_a$ VIA LANGMUIR ISOTHERM;
$k_a$ VIA CONTINUOUS STREAMING DEVICE

$\text{[11]}$

$k_a = \frac{4 \pi l_0^2 D F(N/1000)}{(N = \text{Avogadro's } \# = 6.02 \times 10^{23})}$

$\text{[12]}$

$F (\text{von Smoluchowski, 1918})$

$F = \int_{\varphi} \exp \left[ -\frac{1}{l} \int_{l=l_0}^{l=\infty} \left( \frac{\delta G}{kT} \right) dl \right] d\varphi$

$l = \text{distance}

l_0 = \text{minimum equilibrium distance} = 0.157 \text{ nm}

D = \text{diffusion constant of (e.g.) Ab molecule}

\varphi = \text{orientations of Ab molecules}$
Figure 13.3  Schematic diagram of the adsorption/desorption device.
Tables of $k_a$, $k_d$ and $K_a$
values show slight variability of $k_a$ and strong variability of $k_d$.

From experimental data on haptene-Ab interactions and on the adsorption of human serum albumin onto silica particles it became clear that $k_a$ is fairly constant but that $k_d$ expresses the variabilities inherent in the different systems.

$K_a$ values only express:

$$K_a = \frac{k_a}{k_d} \quad [\text{Eq. 5}]$$

But can be measured independently.)
<table>
<thead>
<tr>
<th>Antibody</th>
<th>Hapten</th>
<th>$k_a$</th>
<th>$k_d$</th>
<th>$K_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit anti-DNP</td>
<td>DNP-lysine</td>
<td>$8.4 \times 10^7$</td>
<td>11</td>
<td>$7.6 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>DNP-lysine</td>
<td>$1.1 \times 10^7$</td>
<td>0.5</td>
<td>$2.2 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>DNP-lysine</td>
<td>$1.3 \times 10^8$</td>
<td>53</td>
<td>$2.5 \times 10^6$</td>
</tr>
<tr>
<td>Rabbit anti-DNP</td>
<td>DNP-glycine</td>
<td>$1.9 \times 10^8$</td>
<td>1300</td>
<td>$1.5 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>DNP-aminocaproate</td>
<td>$9.7 \times 10^7$</td>
<td>1.1</td>
<td>$8.8 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>DNP-aminocaproate</td>
<td>$8.0 \times 10^7$</td>
<td>8.7</td>
<td>$9.2 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>TNP-aminocaproate</td>
<td>$4.0 \times 10^7$</td>
<td>27.0</td>
<td>$1.5 \times 10^6$</td>
</tr>
<tr>
<td>1N-3,6S-2-DNP</td>
<td>$8.0 \times 10^7$</td>
<td>1.4</td>
<td></td>
<td>$5.7 \times 10^7$</td>
</tr>
<tr>
<td>1N-2,5S-4-DNP</td>
<td>$9.5 \times 10^6$</td>
<td>76</td>
<td></td>
<td>$1.3 \times 10^6$</td>
</tr>
<tr>
<td>1N-2,5S-4-DNP</td>
<td>$1.6 \times 10^7$</td>
<td>80</td>
<td></td>
<td>$2.0 \times 10^5$</td>
</tr>
<tr>
<td>1N-2.5S-4-DNP</td>
<td>$1.4 \times 10^7$</td>
<td>410</td>
<td></td>
<td>$3.4 \times 10^4$</td>
</tr>
<tr>
<td>Rabbit anti-TNP</td>
<td>TNP-aminocaproate</td>
<td>$9.0 \times 10^7$</td>
<td>1.6</td>
<td>$5.6 \times 10^7$</td>
</tr>
</tbody>
</table>

$k_a$ varies from $9.5 \times 10^6$ to $1.9 \times 10^8$ L/Msec = 20 fold
$k_d$ varies from 0.5 to 1300 sec$^{-1}$ = 2,600 fold
$K_a$ varies from $3.4 \times 10^4$ to $8.8 \times 10^7$ L/M = 2,588 fold

Determination of the specific adsorption rate constant, $k_a^{\text{mic}}$ from $\chi^{\text{mic}}$ (1-a) and the $k_a$ constant for HSA at $f=1$. Also given are the equilibrium constants, $K_{eq}^{\text{mic}}$ and $k_{d}^{\text{mic}}$, obtained from $k_a^{\text{mic}}$ and $K_{eq}$.

<table>
<thead>
<tr>
<th>System:</th>
<th>$0.4065\chi^{\text{mic}}$</th>
<th>$f^{\text{mic}} = \exp(-0.4065\chi^{\text{mic}})$</th>
<th>$k_a^{\text{mic}} = 7.13 \times 10^{-7} f^{\text{mic}}$</th>
<th>$K_{eq}^{t-&gt;0}$ (L/M)</th>
<th>$k_{d}^{\text{mic}} = k_a^{\text{mic}} / K_{eq}^{t-&gt;0}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSA and</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polysized Silica</td>
<td>-1.203</td>
<td>3.33</td>
<td>2.37$x10^8$</td>
<td>4.11$x10^7$</td>
<td>5.77</td>
</tr>
<tr>
<td>Monosized Silica</td>
<td>-1.203</td>
<td>3.73</td>
<td>2.66$x10^8$</td>
<td>2.78$x10^8$</td>
<td>0.96</td>
</tr>
<tr>
<td>Monosized COO$^-$ silica</td>
<td>-0.817</td>
<td>2.26</td>
<td>1.61$x10^8$</td>
<td>4.2$x10^6$</td>
<td>38.33</td>
</tr>
<tr>
<td>Polysized Talc</td>
<td>-1.126</td>
<td>3.08</td>
<td>2.20$x10^8$</td>
<td>9.7$x10^8$</td>
<td>0.23</td>
</tr>
</tbody>
</table>

$k_a$ varies from $1.61 \times 10^8$ to $2.66 \times 10^8$ L/M.sec = 1.65 fold

$k_d$ varies from 0.23 to 38.3 sec$^{-1}$ = 167 fold

$K_{eq}$ varies from $4.2 \times 10^6$ to $9.7 \times 10^8$ L/M = 230 fold