

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:

$$K_a = k_a/k_d$$

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 ( $\approx 1,000$  SPECIFICITIES)

ADAPTIVE: SPECIFIC BIOPOLYMERS ONE FORMS THROUGH ENCOUNTER. AFTER BIRTH ( $\approx 1,000,000$  SPECIFICITIES)

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

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

ADAPTIVE: ANTIBODIES  
(I.E.: IMMUNOGLOBULINS)  
(MADE BY B-CELLS)

FURTHERMORE, THERE ARE MANY INNATE HELPER PROTEINS, SERVING, E.G., AS MESSAGERS TO ACTIVATE PHAGOCYTOTIC AND OTHER KILLER CELLS.

EXAMPLES:

COMPLEMENT FACTORS,  
OTHER CYTOKINES

CELLS

PHAGOCYTOTIC CELLS:

1<sup>ST</sup> LINE OF DEFENCE { POLYMORPHONUCLEAR CELLS,  
ESPECIALLY NEUTROPHILS

2<sup>ND</sup> LINE OF DEFENCE { MONOCYTES, WHICH BECOME  
MACROPHAGES

KILLER CELLS

LYMPHOCYTES { T CELLS  
NK (natural killer) CELLS

**ANTIBODIES: ADAPTIVE!**

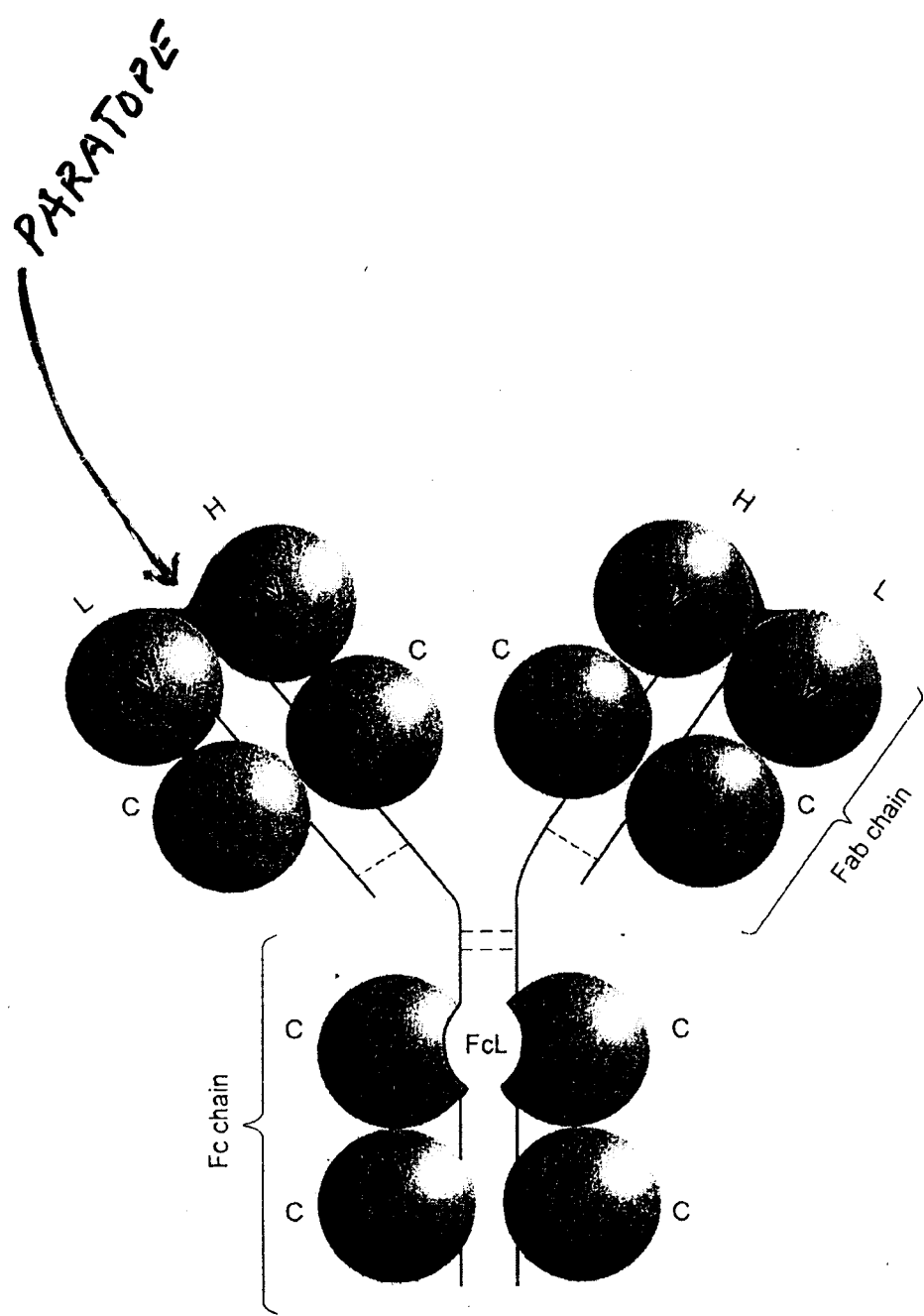
ANTIBODIES ARE IMMUNOGLOBULINS; IN HUMANS:

SERUM %

	Ig M, Ig G, Ig A, Ig E, Ig D
	10      2      2      2      2
	← VALENCIES
≈ 0.1	Ig M: Mw ≈ 900,000, PRIMARY RESPONSE
≈ 1.2	Ig G: " ≈ 150,000, SECONDARY RESP.
≈ 0.25	Ig A: " ≈ 155,000, SECRETORY AB'S
≈ 0.0001	Ig E: " ≈ 185,000, ALLERGIES; ANTI-PARASITE
≈ 0.003	Ig D: " ≈ 187,000, NEWBORNS; PRE-PRIMARY

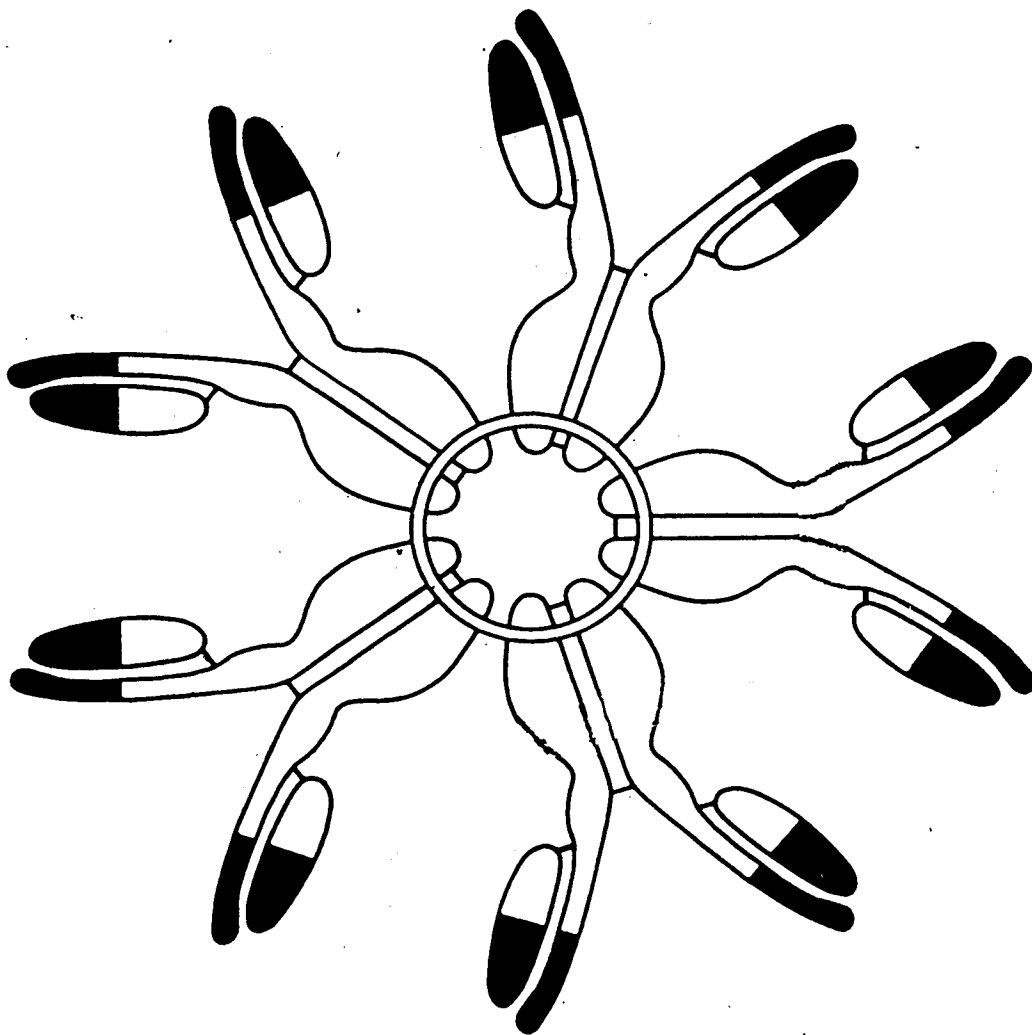
AFTER FIRST ENCOUNTER WITH NEW Ag, AB'S TAKE ≈ ONE WEEK TO DEVELOP; AFTER SECOND ENCOUNTER: MUCH FASTER. TOTAL # OF SPECIFICITIES: ≈ 1,000,000

Ig G IS THE ONLY Ig THAT PASSES THE PLACENTA; HALF-LIFE OF Ig G ≈ 3 WEEKS



IgG

← 100 Å →



IgM

# ANTIGENS

BIOPOLYMERS: Most frequently:

PROTEINS  
GLYCOPROTEINS

ALSO: GLUCIDES (E.G.:  
Blood group sugars)

DNA (Only patho-  
logically)

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

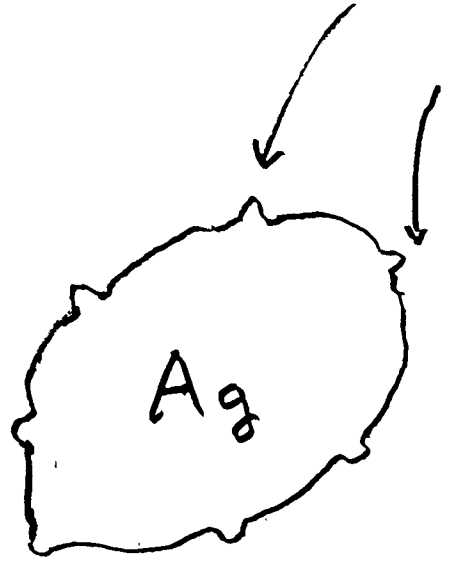
INACTIVE:

HYDROPHOBIC: Polybutadiene, polystyrene,  
silicones

HYDROPHILIC: PEO, polyvinyl alcohol, poly-  
vinyl toluene, polyacrylic  
acid, polystyrene-sulphonic  
acid



EPITOPES



# Ag-Ab INTERACTIONS - 1

OCCUR VIA THE:

ANTIGEN-SPECIFIC SITE, OR:

EPITOPE

PLUS THE ANTIBODY-ACTIVE

SITE, OR:

PARATOPE

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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

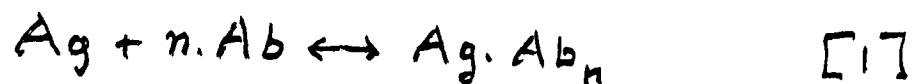
# Ag-Ab INTERACTIONS - 2

## ENERGETICS

V

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:



THEN:

$$K_a = \frac{[Ag \cdot Ab_n]}{[Ag] \cdot [Ab]^n} \quad [2]$$

FURTHERMORE:  $\frac{\overset{(mJ/m^2)}{\Delta G_{iw2}} \times S_c}{kT} = \overset{(kT)}{\Delta G_{iw2}^*} \quad [3]$

AND:  $\Delta G_{iw2} \text{ (in } kT) = -\ln(K_a \times 55.6) \quad [4]$

## Ag-Ab INTERACTIONS-3

### ENERGETICS & POLYCLONAL VS. MONOCLONAL ANTIBODIES

UP TO THE END OF THE 20<sup>TH</sup> CENTURY ONE ONLY HAD POLYCLONAL AB'S + PLURIVALENT Ag's, RESULTING IN Ag-Ab COMPLEXES OF MIXED Ag/Ab RATIOS LEADING TO A NON-SOLVABLE:

$$K_a = \frac{[Ag_n - Ab_m]}{[Ag]^n \cdot [Ab]^m} \quad [2-bis]$$

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'S BECAME POSSIBLE

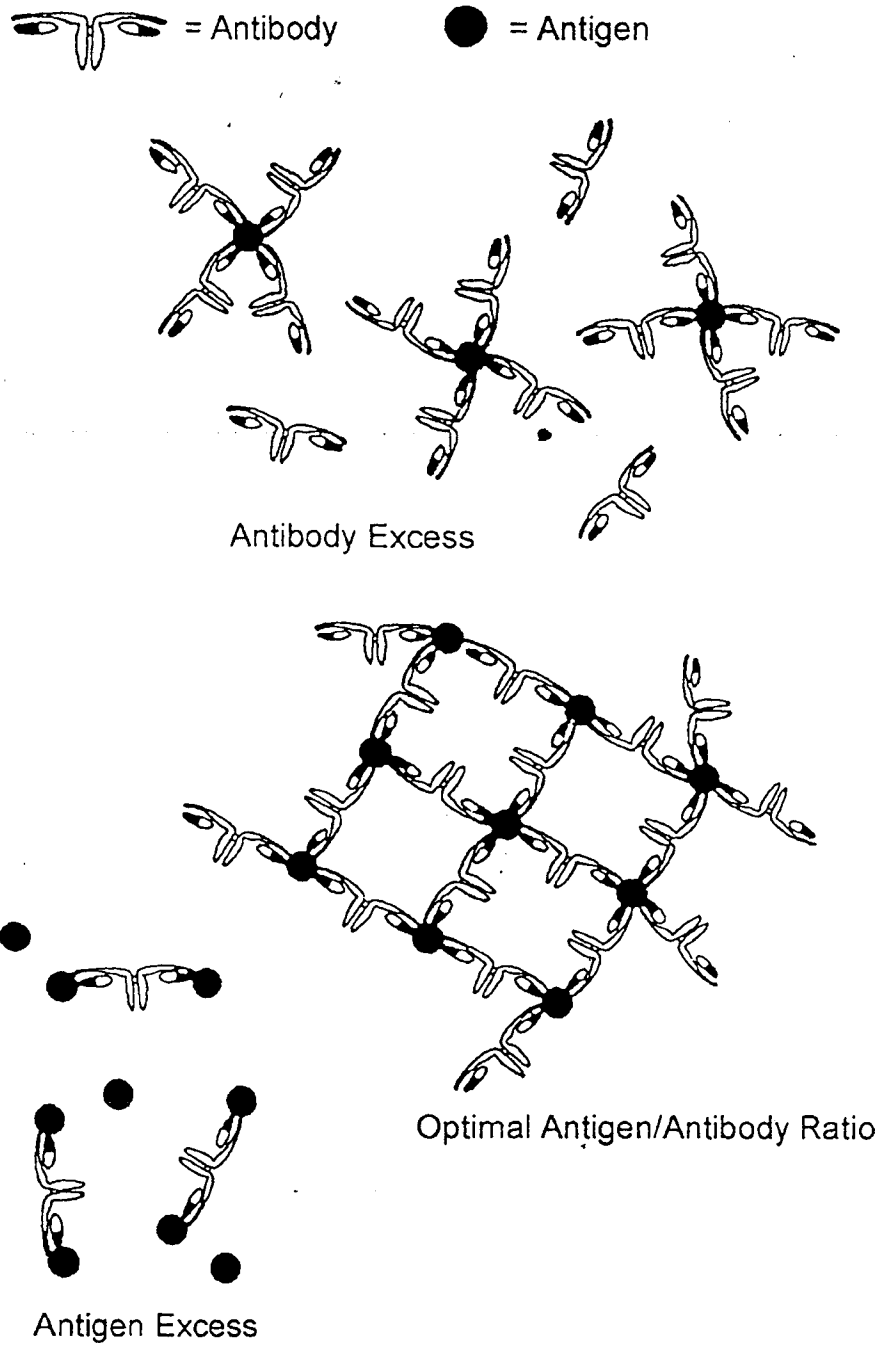
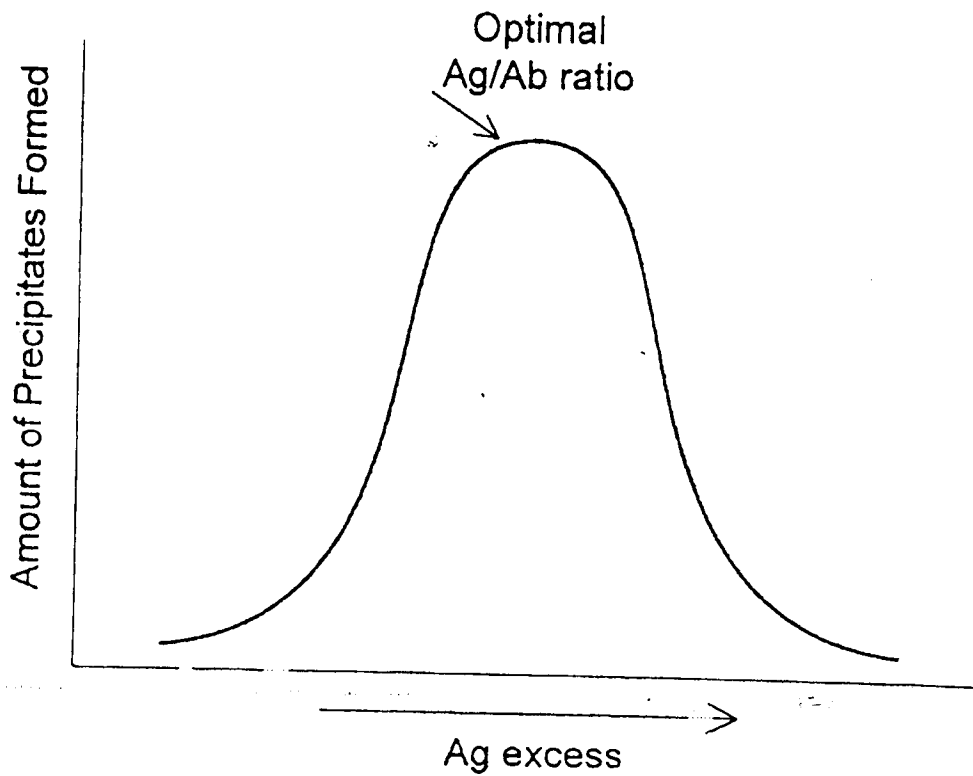


Figure 7.4 : Composition of three typical Ag-Ab complexes at different Ag/Ab ratios.



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 ( $\Delta G_t^{LW}$ ) and forces of interaction of ( $F_t^{LW}$ ) of unretarded Lifshitz-van der Waals interaction, for a number of configurations, as a function of distance,  $\ell$

Configuration	$\Delta G_t^{LW}$	$F_t^{LW}$
(semi-infinite flat parallel slabs)	$-\frac{A}{12\pi\ell^2}$	$\frac{A}{12\pi\ell^3}$
(sphere of radius R and semi-infinite flat slab; also valid for two crossed cylinders at 90°)	$-\frac{AR}{6\ell}$	$\frac{AR}{6\ell^2}$
(two spheres of radius R)	$-\frac{AR}{12\ell}$	$\frac{AR}{12\ell^2}$

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

Configuration	$\Delta G_t^{AB}$	$F_t^{AB}$
(flat parallel plates)	$\Delta G_{\ell_0}^{AB''} \exp[(\ell_0 - \ell)/\lambda]$	$F_{\ell_0}^{AB''} \exp[(\ell_0 - \ell)/\lambda]$ $= -\Delta G_{\ell_0}^{AB''} (1/\lambda) \exp[(\ell_0 - \ell)/\lambda]$
(sphere of radius R and flat plate; also valid for two crossed cylinders at 90°)	$2\pi R \lambda \Delta G_{\ell_0}^{AB''} \exp[(\ell_0 - \ell)/\lambda]$	$-2\pi R \Delta G_{\ell_0}^{AB''} \exp[(\ell_0 - \ell)/\lambda]$
(two spheres of radius R)	$\pi R \lambda \Delta G_{\ell_0}^{AB''} \exp[(\ell_0 - \ell)/\lambda]$	$-\pi R \Delta G_{\ell_0}^{AB''} \exp[(\ell_0 - \ell)/\lambda]$

<sup>a</sup> $\Delta G_{\ell_0}^{AB''}$  is obtained from eqs. [III-6], [III-16], or [III-17], and  $F_{\ell_0}^{AB''} \approx -(1/\lambda) \Delta G_{\ell_0}^{AB''}$ , or  $F_t^{AB}$  is measured experimental. The superscript " indicates that  $\Delta G''$  or  $F''$  were obtained at the plane parallel plate configuration, at the minimum equilibrium distance  $\ell_0$ .



**TABLE VI-3** Energies of interaction ( $G_t^{EL}$ ) and forces of interactions ( $F_t^{EL}$ ) of electrostatic interactions, for a number of configurations, as a function of distance,  $\ell^a$ , for relatively weak interactions, i.e., for  $\zeta < 25$  mV

Configuration	$\Delta G_t^{EL}$	$F_t^{EL}$
(flat parallel plates)	$1/\kappa \cdot 64nkT\gamma_0^2 \exp(-\kappa\ell)^b$	$-64nkT\gamma_0^2 \exp(-\kappa\ell)^b$
(sphere of radius R and flat plate; also valid for two crossed cylinders at 90°)	$\epsilon R \psi_0^2 \ln[1 + \exp(-\kappa\ell)]$	$-\epsilon \kappa R \psi_0^2 \ln[1 + \exp(-\kappa\ell)]$
(two spheres of radius R)	$0.5\epsilon R \psi_0^2 \ln[1 + \exp(-\kappa\ell)]$	$-0.5\epsilon \kappa R \psi_0^2 \ln[1 + \exp(-\kappa\ell)]$

<sup>a</sup>For explanation of the symbols used, see Chapter IV, eqs. [IV-2-IV-6].

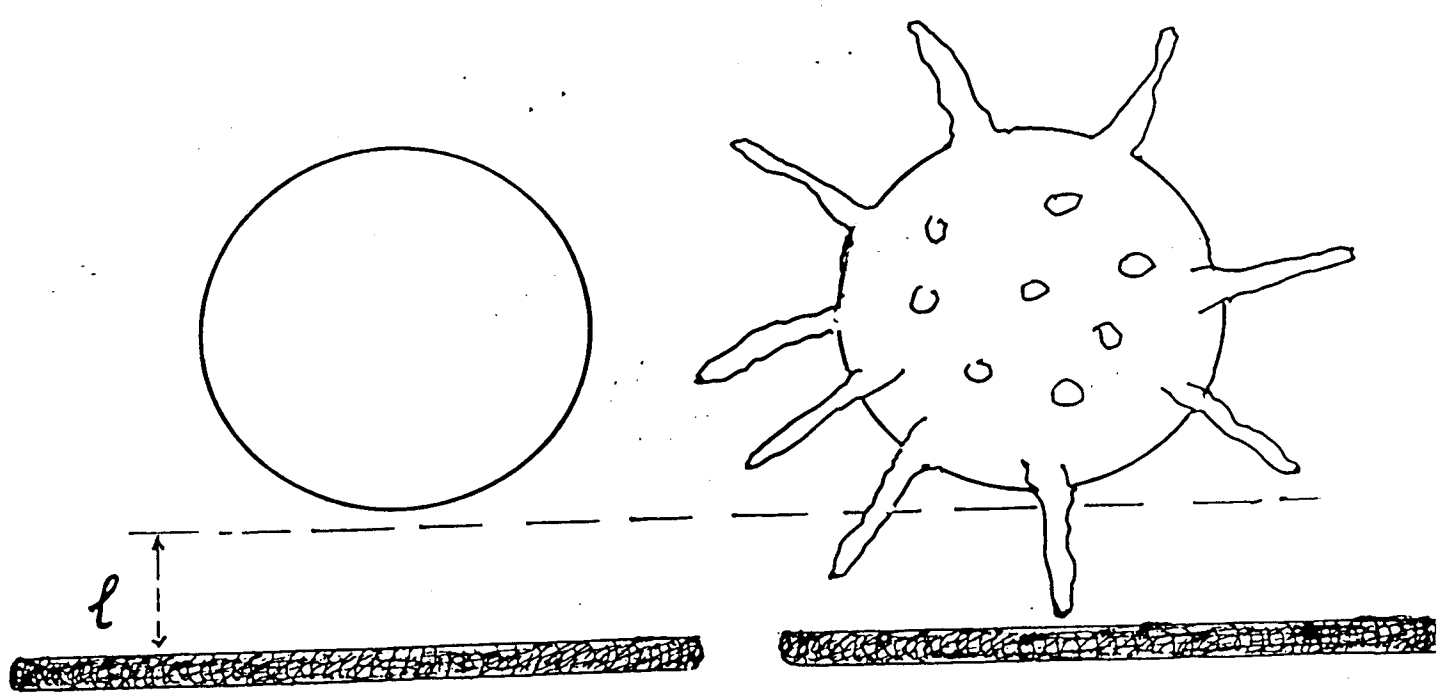
<sup>b</sup> $\gamma_0 = [\exp(v\psi_0/2kT) - 1]/[\exp(v\psi_0/2kT) + 1]$  (eq. [IV-4]).

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])\*

	$\Delta G_{\text{S-S}}^{\text{VW}} =$	$\Delta G_{\text{S-S}}^{\text{AB}} =$	$\Delta G_{\text{S-P}}^{\text{EL}} =$
	$-AR/12\ell^2$	$\pi R \lambda \Delta G_{\text{S-S}}^{\text{AB}} \exp [(\ell_0 - \ell)/\lambda]^b$	$0.5 \epsilon R \psi_0^2 \ell \ln [1 + \exp(-\kappa \ell)]^c$
	$-AR/6\ell^2$	$2\pi R \lambda \Delta G_{\text{S-S}}^{\text{AB}} \exp [(\ell_0 - \ell)/\lambda]^b$	$\epsilon R \psi_0^2 \ell \ln [1 + \exp(-\kappa \ell)]^c$

EXTENDED DLVO THEORY;  
 INFLUENCE OF  
 R





A

B

# KINETICS-1

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

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

$$k_a \text{ in L/(Msec)} \quad [7]$$

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

$$K_d = \frac{k_d}{k_a} = \frac{1}{K_a} \quad [9]$$

- $K_d$  AND  $k_d$  CANNOT BE MEASURED DIRECTLY DUE TO HYSTERESIS
- NEVER USE  $K_d$  !
- DETERMINE  $k_d$  VIA:  $k_d = \frac{k_a}{K_a}$  [10]

# KINETICS-2

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

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

$$k_a = 4\pi l_0 D F (N/1000)$$

( $N = \text{Avogadro's } \# = 6.02 \times 10^{23}$ )

[11]

and:  $F$  (VON SMOLUCHOWSKI, 1918)

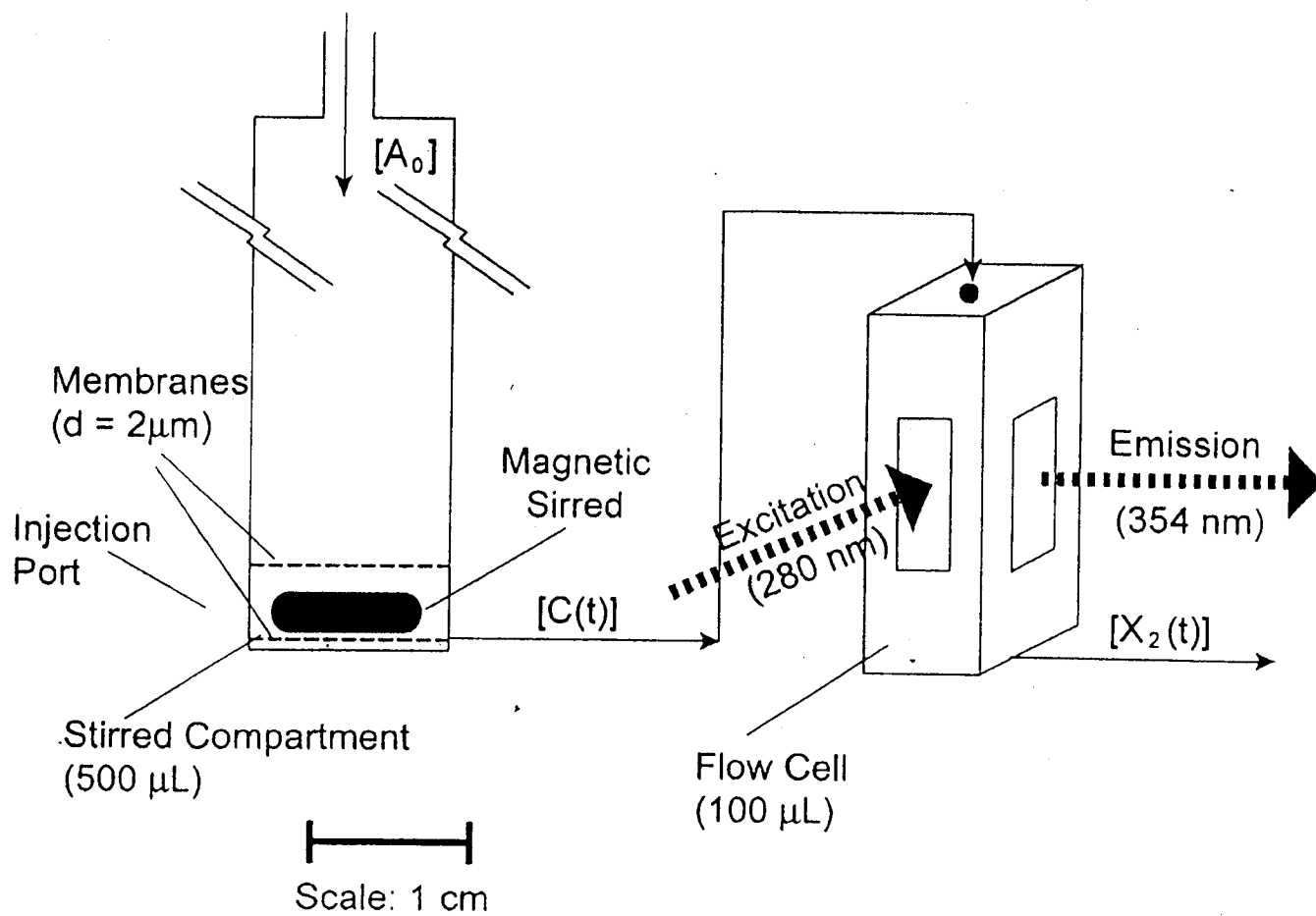
$$F = \int \exp \left[ \frac{1}{l} \int_{l=l_0}^{l=\infty} \left( \frac{\Delta G}{kT} \right) dl \right] d\varphi \quad [12]$$

$l$  = distance

$l_0$  = minimum equilibrium distance = 0.157 nm

$D$  = diffusion constant of (e.g.) Ab molecule

$\varphi$  = 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 VARIA-  
BILITY OF  $k_a$  AND STRONG  
VARIABILITY OF  $k_d$ :

FROM EXPERIMENTAL DATA  
ON HAPTEN-AB INTER-  
ACTIONS AND ON THE  
ADSORPTION OF HUMAN  
SERUM ALBUMIN ONTO  
SILICA PARTICLES IT BE-  
CAME 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)

TYPICAL KINETIC DATA FOR SOME SELECTED HAPTEN-ANTIBODY REACTIONS \*, \*\*

Antibody	Hapten	$k_a$	$k_d$	$K_a$
Rabbit anti-DNP	DNP-lysine	$8.4 \times 10^7$	11	$7.6 \times 10^7$
Mouse anti-DNP	DNP-lysine	$1.1 \times 10^7$	0.5	$2.2 \times 10^7$
	DNP-lysine	$1.3 \times 10^8$	53	$2.5 \times 10^6$
Rabbit anti-DNP	DNP-glycine	$1.9 \times 10^8$	1300	$1.5 \times 10^5$
	DNP-aminocaproate	$9.7 \times 10^7$	1.1	$8.8 \times 10^7$
	DNP-aminocaproate	$8.0 \times 10^7$	8.7	$9.2 \times 10^6$
	TNP-aminocaproate	$4.0 \times 10^7$	27.0	$1.5 \times 10^6$
	1N-3,6S-2-DNP	$8.0 \times 10^7$	1.4	$5.7 \times 10^7$
	1N-2,5S-4-DNP	$9.5 \times 10^6$	76	$1.3 \times 10^6$
	1N-2,5S-4-DNP	$1.6 \times 10^7$	80	$2.0 \times 10^5$
	1N-2,5S-4-DNP	$1.4 \times 10^7$	410	$3.4 \times 10^4$
Rabbit anti-TNP	TNP-aminocaproate	$9.0 \times 10^7$	1.6	$5.6 \times 10^7$

\* $k_a$  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  $\text{sec}^{-1}$  = 2,600 fold  
 $K_a$  varies from  $3.4 \times 10^4$  to  $8.8 \times 10^7$  L/M = 2,588 fold

\*\*From D.R. Absolom and C.J. van Oss, Crit. Rev. Immunol. 6(1986)1. See also A.Froese, Immunochemistry 5(1968)253.

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,  $K_{\text{eq}}$ , and  $k_d^{\text{mic}}$ , obtained from  $k_a^{\text{mic}}$  and  $K_{\text{eq}}$

System:	$0.4065\chi^{\text{mic}}$ (kT)	$f^{\text{mic}} =$ $\exp(-0.4065\chi^{\text{mic}})$	$k_a^{\text{mic}} =$ $7.13 \times 10^7 f^{\text{mic}}$	$K_{\text{eq}}^{t \rightarrow 0}$ (L/M)	$k_d^{\text{mic}} = k_a^{\text{mic}} / K_{\text{eq}}^{t \rightarrow 0}$ (s <sup>-1</sup> )
Polysized Silica	-1.203	3.33	$2.37 \times 10^8$	$4.11 \times 10^7$	5.77
Monosized Silica	-1.203	3.73	$2.66 \times 10^8$	$2.78 \times 10^8$	0.96
Monosized COO <sup>-</sup> silica	-0.817	2.26	$1.61 \times 10^8$	$4.2 \times 10^6$	38.33
Polysized Talc	-1.126	3.08	$2.20 \times 10^8$	$9.7 \times 10^8$	0.23

$k_a$  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<sup>-1</sup> = 167 fold

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

From: A. Docoslis, W.Wu, R.F. Giese, C.J. van Oss, Colloids Surfaces-B 25 (2002) 97;

see also: Colloids Surfaces-B 22 (2001) 205.