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PHYSICAL BASIS *of* CELL-CELL ADHESION

Pierre Bongrand

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Physical Basis of Cell-Cell Adhesion

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INTRODUCTION

Cell adhesion is a ubiquitous process that influences many aspects of cell behavior. Indeed, the control of cell proliferation[1] and migration[2] through different tissues involves adhesive interactions. The invasion of a specific organ by a bacterium[3] or the penetration of a target cell by a virus[4] are initiated by adhesive recognition. The triggering of effector functions such as particle engulfment by a phagocyte[5] or target cell destruction by a cytotoxic T-lymphocyte[6] involves a binding step. Finally, metastasis formation by tumor cells was often postulated to include attachment and detachment steps, and statistical correlations were demonstrated between the adhesiveness and invasive potential of malignant cells in different experimental systems.[7]

Hence, it is not surprising that many authors studied adhesion on a variety of cellular models, with different experimental methods. In many cases, cell adhesion seemed to be driven by specific receptor-ligand interactions and much work was devoted to the characterization of "adhesion molecules".[8-10] However, several experimental data suggest that an exhaustive study of the structure and specificity of cell adhesion molecules would not allow a complete understanding of adhesion. Here are some specific examples.

Concanavalin A is a molecule with four binding sites specific for carbohydrate residues commonly found on different cell membranes. It is therefore not surprising that many cells are agglutinated by this substance through a cross-bridging mechanism. However, treating cells with glutaraldehyde, a well-known fixation procedure, may induce a drastic decrease of agglutinability without a parallel decrease of concanavalin A-binding ability.[11,12]

It is a common finding in many experimental systems[6] that cell adhesion may be decreased by the addition of divalent cation chelators, inhibitors of cell energy production or cytoskeleton assembly, or temperature decrease. In many cases, it is unlikely that these phenomena reflect a loss of cell surface receptors or alteration of these receptors with concomitant decrease of binding affinity.

Antibody-mediated erythrocyte agglutination is a widely used method of studying the presence of different antigens on the red cell surface. This is indeed a routine technique in blood transfusion centers and it is well known that in some cases antibodies cannot mediate agglutination unless some special procedures (such as modification of ionic strength or protein addition) are used. It is unlikely that these relatively mild procedures act by increasing antigen-antibody affinity.[13]

When cells are coated with low amounts of concanavalin A, they may be agglutinated provided they are subjected to gentle centrifugation. However, prolonged agitation of cell suspensions may not result in adhesion, despite the occurrence of numerous cell-cell encounters.[12]

Another point is that in some situations the concept of specific-bond mediated adhesion does not seem to hold. As an example, many cells may adhere to a variety of synthetic substrates that may hardly be considered as specific ligands for adhesion molecules.[14] Hence, in addition to well-characterized specific interactions, cell adhesion may involve a combination of nonspecific low affinity molecular associations.

Recent experimental and theoretical progress suggests that some results and physical methods may be used to deal with the aforementioned problems. Indeed, physics may help define and measure quantitative parameters of cell adhesion such as kinetics of bond formation, mechanical strength of adhesions, width of the cell-cell or cell-substrate gap in adhesive zones, adhesion-associated strain, and stress of the cell surface. Also, physical techniques may allow a quantitative description of the different cell properties relevant to adhesion, such as surface charge and hydrophobicity or mechanical properties. Finally, physical results obtained by studying model systems may yield some information on the forces experienced by membrane molecules during the cell-cell approach.

The present book is aimed at providing a readable account of physical methods and results required to measure cell adhesion and interpret experimental data. Since on the one hand readability seemed a major quality for a book, and on the other hand, the problems posed referred to a wide range of domains of physics, chemistry, and biology, completeness had to be sacrificed. Indeed, a whole book would not suffice to quote the relevant literature (and many more authors would be required to have read it). Hence, only a limited number of topics were selected for reliability of methods, availability of enough experimental results to illustrate basic concepts or potential use in the future. These were discussed in three sections.

Section I includes a basic physical background likely to help understanding of cell adhesion.

Intermolecular forces are reviewed in the first chapter; after a brief description of the structure of the cell surfaces, molecular interactions are described in systems of increasing complexity, from atoms in vacuum to macroscopic bodies suspended in aqueous ionic solutions. Also, selected examples of interactions between biological macromolecules are reviewed to convey a feeling for the concept of ''binding specificity''.

In Chapter 2, de Gennes gives a description of the latest principles underlying the interactions between polymer-coated surfaces. Although many problems remain unsolved, the language of polymer physics should provide a basically correct framework for the discussion of intercellular forces, since cells are essentially polymer-coated bodies surrounded by solute macromolecules.

In Chapter 3, some methods and results of surface physics are presented, since the wealth of experimental data gathered in this field may shed some light on the mechanisms of interaction between ill-defined surfaces such as cell membranes.

Chapter 4 is devoted to a description of recent results on the mechanical properties of cell membranes. Systematic use and development of the powerful micropipette aspiration technique allowed Dr. Evans to obtain a reliable picture of the cell response to mechanical stimuli. This kind of knowledge is an essential requirement for a correct understanding of the mechanisms by which cell surfaces are deformed to allow the appearance of extended cell-cell or cell-substrate contact areas.

The second section of the book includes a description of some quantitative methods of studying cell adhesion as well as selected experimental results.

Hydrodynamic flow methods are reviewed in Chapter 5. These methods provide a simple way of evaluating the minimal time required for the formation of stable intercellular bonds and the mechanical resistance of these bonds.

As a logical sequel to the description of procedures for generating and breaking intercellular bonds, David Segal presents in Chapter 6 the powerful and versatile methods of detecting and quantifying cell aggregation he developed with a flow cytometer. The increasing availability of this apparatus may make Dr. Segal's methodology a procedure of choice for those interested in the measurement of cell adhesion.

In Chapter 7, Evans describes an analysis of the adhesion-induced deformations he measured in different models. This work allowed quantitative evaluation of the work of adhesion between cell surfaces.

Colette Foa and colleagues present electron microscopical data on cell adhesion and describe a methodology allowing quantitative analysis of digitized micrographs in Chapter 8. Their results demonstrate the difficulty of a physical analysis of the adhesion between ''usual'' cells, since plasma membranes are studded with asperities of varying shape and unknown mechanical properties. A quantitative description of these features is needed to model adhesive processes involving these surfaces.

In Chapter 9, Curtis reviews a variety of experimental studies on cell adhesion with an emphasis on the danger of interpreting the obtained data with simplistic concepts. This shows how much caution is needed when detailed mechanisms are proposed to account for experimental data.

Finally, Chapter 10 is a description by George Bell of some models for the kinetics of intercellular bond formation and equilibrium contact area. It is shown that fairly simple and reasonable assumptions may lead to quite rich models, with predictions that are not excessively dependent on the details of underlying assumptions. These models may help understand available experimental data and suggest further studies.

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Section I
Basic Physical Background

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Chapter 1

INTERMOLECULAR FORCES

Pierre Bongrand

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I. INTRODUCTION

An obvious requirement for understanding cell-cell adhesion is to know what forces are experienced by membrane molecules when two cells encounter each other. Unfortunately, obtaining such knowledge seems a formidable task. Indeed, the detailed molecular structure of cell membranes has not yet been completely determined, although much progress was achieved during the last few years. Further, even if all required data were available, there would remain a need to predict the interaction between a very wide range of complex structures. This is by no means a simple problem since even the interaction between two small atoms or molecules in biological media is not easily amenable to rigorous theoretical treatment. The last point may be exemplified by the following data: the electronic energy of a small atom such as ^{12}C is on the order of 10^{-17} to 10^{-16} J.[1] The energy of a typical covalent bond such as C–C is much smaller since it is about 6×10^{-19} J.[2] However, intercellular adhesion usually involves a large number of much weaker noncovalent interactions; the energy of a typical hydrogen bond in vacuum is about 3×10^{-20} J.[3] Finally, in biological media, interactions between cell-surface molecules are often substantially screened

by competition with water or ions. For example, this competition may decrease the energy of a hydrogen bond by a factor of 10.[4] Hence, the binding energies that are of interest for us are differences between much higher quantities. As a consequence, theoretical determination of intermolecular forces involves a cascade of calculations and approximations, which makes it very difficult to assess the validity of obtained results. Further, as is discussed later in the chapter, the experimental determination of binding energies is not a straightforward process and may also involve many assumptions. This situation is responsible for the many errors and unwarranted assumptions that appeared during the history of the study of intermolecular forces.[5]

The purpose of the present review is to provide a brief sketch of the available methods (both experimental and theoretical) of studying intermolecular forces that were applied to biological adhesion or that seemed of potential interest in this domain. Selected experimental results are also presented. First, a molecular description of the cell surface is given, in order to provide the reader with a well-defined model for applying different methods and results on intermolecular forces. Second, forces between atoms and molecules are described, both in vacuum and in aqueous ionic solutions. Third, forces between macroscopic bodies are discussed; although these are a sum of interatomic forces, specific theories were elaborated to bypass a detailed account of individual molecules. Fourth, selected examples of molecular interactions likely to occur near cell surfaces are described.

II. MOLECULAR STRUCTURE OF THE CELL SURFACE

Our aim is to provide a basically correct (although admittedly approximate) view of intermolecular forces that are likely to be generated when the distance between two cell surfaces is decreased. For this purpose, the cell surface has to be described at the nanometer level. Additional information on the organization of cell asperities with a size on the order of 0.1 to 1 μm may be found in Chapter 8. Also, much information of the mechanical properties of cell surfaces (as studied at the micrometer level) may be found in Chapter 4.

A. Core Structure of the Cell Plasma Membrane

The "fluid mosaic" model of the cell membrane as elaborated by Singer and Nicolson[6] is now widely accepted. More recent information may be found in excellent textbooks.[7,8] The basic structure is made of a lipid bilayer tightly associated to intrinsic membrane proteins (Figure 1).

1. Lipids

The lipid bilayer is made of amphipathic molecules comprising phospholipids (e.g., phosphatidylcholine or sphingomyelin), cholesterol, and glycolipids (such as gangliosides). This structure comprises a hydrophobic middle layer of about 40 Å thickness[9] that is made of hydrocarbon chains, and two external hydrophilic layers of about 15 Å thickness each[9]; these include the polar heads of lipid molecules and bear electric charges (e.g., the positive charges of choline groups and the negative charges of phosphate groups or the sialic-acid residues of gangliosides). It is well known that the bilayer is in a fluid state[10] and lipid molecules exhibit thermal motion with a diffusion constant on the order of 10^{-8} cm^2/sec.[11] However, molecular associations may result in coordinated movements of subclasses of lipid molecules (e.g., gathering of gangliosides to a pole of the cell after suitable cross-linking of a fraction of these molecules, a process called capping[12]). This possibility may be relevant to cell adhesion since local redistribution of membrane molecules may substantially alter intercellular forces. A final point is that several reports emphasized the possibility of phase separation with formation of distinct lipid domains.[13]

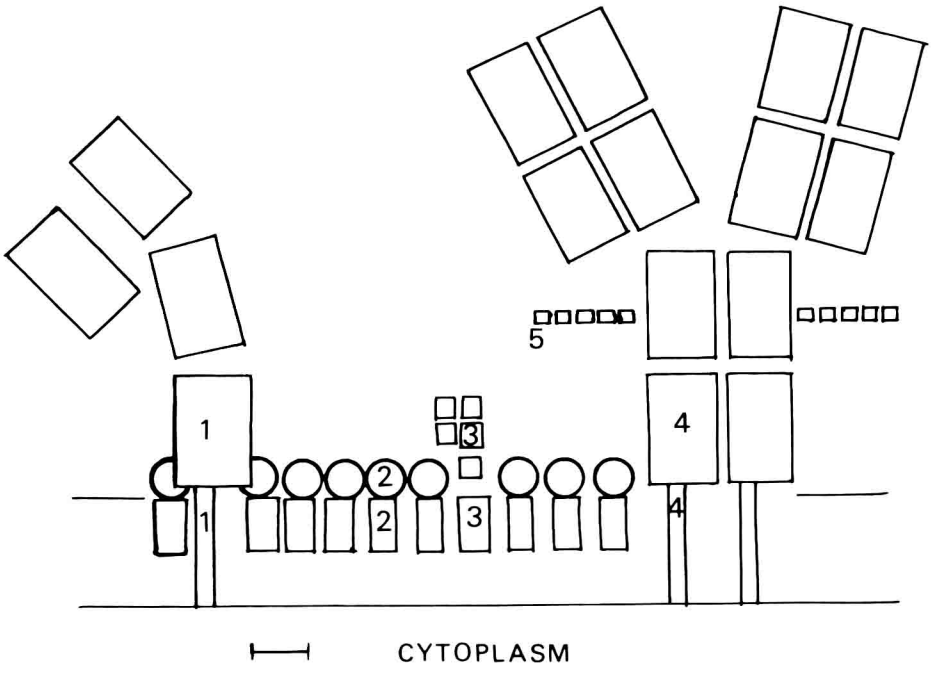

FIGURE 1. Molecular shape of the cell surface at the nanometer level. Several molecules found on cell surfaces are shown. Emphasis is on molecular size rather than precise conformation. (1) Class I histocompatibility molecule, (2) phospholipid molecule, (3) ganglioside GM1, (4) surface immunoglobulin G (some lymphoid cells), (5) oligosaccharide chain. Bar is 2 nm.

2. Proteins

Cell-surface lipids are bound to a similar amount (by weight) of so-called "intrinsic membrane proteins" that cannot be removed without the aid of detergents and concomitant disorganization of the bilayer structure.[14] An erythrocyte membrane contains about 5×10^6 polypeptide chains[14] for a total area of about 140 μm^2.[15] A small number of protein species accounts for most of the membrane protein content; an erythrocyte membrane contains about 500,000 glycophorin molecules.[7] Lymphoid cells are endowed with several hundreds of thousands of class I histocompatibility molecules.[17] Also, many functionally important proteins are much less numerous on the cell surface (e.g., transport proteins, ionic channels, or hormone receptors).

Although the structure of membrane proteins is more difficult to study than that of soluble molecules, sufficient information is available to construct a reasonable picture of the cell-surface intrinsic proteins. Typical molecules may be anchored in the hydrophobic plasma membrane through a sequence of 10 to 20 hydrophobic amino acids,[17,18] and the largest portion is usually exposed to the extracellular aqueous medium (mostly intracellular proteins are of lesser importance for our purpose). Following are some specific examples.

A glycophorin molecule is made of 131 amino acid residues and about 100 saccharide residues arranged as 16 short carbohydrate chains with a length of order of 1 nm each.

The surface of a B lymphocyte may bear sIgM molecules that are made of four polypeptide chains with a molecular weight of about 180,000 (with 10% carbohydrate). The amino acid residues form 14 globular domains of 20 to 25 Å diameter. The molecule protrudes above the bilayer surface by about 100 Å. This structure has long been determined due to the easy availability of large amounts of soluble immunoglobulin molecules.

Class I histocompatibility molecules are made of "heavy" polypeptide chains of about 44,000 mol wt and β2 microglobulin chains of 12,000 mol wt. The discovery of sequence

homologies between these proteins and immunoglobulins makes attractive the concept of an immunoglobulin-like domain structure for histocompatibility molecules. These molecules might thus be viewed as fairly globular entities of about 50 Å size. This example is important since essentially all nucleated cells bear histocompatibility molecules on their surface.

An additional result was obtained by electron microscopic observation of carbon or platinum replica obtained after freeze-fracture of plasma membranes, a procedure that may separate lipid monolayers and expose tangential membrane sections. These sections were found to contain particulate structures of about 75 Å diameter (the so-called intercalated particles) that are thought to represent membrane proteins. An erythrocyte membrane contains about 500,000 of these particles,[8] which is consistent with the view that they are made of a small number of polypeptide chains.

The above data are also consistent with several studies made on the surface distribution of different cell-surface molecules. Ferritin-labeled antibodies were used to label surface immunoglobulins[20] or histocompatibility antigens,[21] and electron micrographs were used to determine the radial distribution function of labeled molecules (in analogy with radial distribution functions used in liquid state theory). These experiments supported the concept that membrane molecules are scattered over the entire cell surface (polarized cells with specialized membrane areas, such as thyroid cells, may display different features[22]). However, the above results refer to undisturbed cells. Different experiments are required to understand the molecular redistribution phenomena that may occur during cell adhesion.

3. Movements of Cell-Surface Proteins

That cell membrane proteins can display extensive in-plane movements that has been checked experimentally for more than 10 years. Frye and Edidin[23] fused human and murine cells after labeling surface antigens with fluorescent antibodies of different colors; heterokaryons displayed rapid mixing of both labels. More recently, the diffusion constant of cell-surface molecules was quantified by measuring fluorescence recovery after photobleaching.[24,25] Briefly, a single cell bearing a fluorescent label bound to some class of membrane molecules is locally illuminated with a laser beam of a fraction of a micrometer width, and fluorescence is measured with a photomultiplier. The labeled molecules located in the illuminated area are then bleached by brief 1000-fold increase of the light intensity. The fluorescence recovery is then monitored under normal illumination. This recovery is ascribed to the replacement of bleached molecules with peripheral ones, due to diffusive movements. Simple quantitative models yielded diffusion coefficients on the order of 10^{-12} to 10^{-11} cm^2/sec. However, fluorescence recovery was often lower than 100%, indicating that a proportion of surface molecules did not exhibit long-range diffusion. Also, molecular diffusion was found to be anisotropic,[26] with a possible difference of a factor of 10 between the diffusion rates of a given molecule when measured along two perpendicular directions. Of course, an underlying assumption was that molecular movements were not altered by the bleaching process.

Another important point is that cross linking of a class of cell-surface molecules may result in extensive redistribution of these molecules with: (1) gathering into small patches scattered on the membrane (the patching phenomenon[27]), (2) gathering into a cap on a pole of the cell (this is the capping phenomenon, an active cellular process[27]), or (3) immobilization of cross-linked molecules.[28] These movements involve interactions between submembranous cytoskeletal constituents and cell-surface molecules.[29] Obviously, these phenomena may play a role in the reorganization of membrane contact areas during intercellular adhesion.

A final point is that redistribution of cell-surface molecules may be induced by a suitable external force. Subjecting embryonic muscle cells to an electric field higher than about 1 V/cm resulted in substantial redistribution of concanavalin A binding structures.[30]

The above data on the organization of intrinsic membrane components are summarized

in Figure 1. However, this figure is not complete due to the presence on the surface of most cell species of more loosely bound polysaccharidic and proteic components collectively known as the cell "glycocalyx" or "fuzzy coat" that we shall now describe.

B. Structure of the Cell Glycocalyx

The word glycocalyx was coined by Bennett in 1963 to denote a polysaccharide-rich cell-surface structure that may be found on most cell species.[31] This may be visualized by staining cells with colloidal iron or ruthenium red and appears as a fuzzy coat the thickness of which may reach 1000 Å or more.[32,33]

Major constituents of the glycocalyx are proteoglycans. A typical proteoglycan such as bovine cartilage proteoglycan is made of a core protein (200,000 mol wt) bearing about 100 glycosaminoglycan chains of about 20,000 mol wt.[34] The length of a proteoglycan molecule may be as high as several micrometers.[35] Glycosaminoglycans are made up of disaccharide repeating units including a derivative of an aminosugar and at least a negatively charged carboxyl or sulfate group.[35]

In a quantitative study made on radioactive sulfate incorporation by fibroblastic cells, Roblin and colleagues[36] found that a typical 3T3 cell incorporated circa 10^{11} to 10^{12} sulfate groups and released this quantity within about 12 to 24 hr.

The cell coat also contains peripheral glycoproteins such as fibronectin. This cell adhesion molecule (also called LETS or cold insoluble globulin) is a glycoprotein (about 5% carbohydrate) made of two polypeptide chains of 225,000 mol wt each, appearing as two strands of 610 Å length and 20 Å diameter, with a limited flexibility restricted to specific regions.[37]

An important point is the relationship between the cell and the cell coat. First, measurement of precursor incorporation[33,36,38] demonstrated the endogenous origin of at least part of this coat. Second, detachment of the cell coat may be spontaneous,[36,39] or obtained by a mere washing[40] or mild exposure to proteolytic enzymes.[36] Hence, there must exist a wide heterogeneity in the tightness of association between the core membrane and the fuzzy coat. Third, peripheral cell macromolecules may be functionally associated with cytoskeletal elements.[41,42]

C. Approximate Quantitative Model of the Cell Surface

Now a realistic model of the cell surface must be built in order to evaluate the importance of the interactions that shall be reviewed in the next section. Due to the occurrence of wide differences between the surfaces of different cell types, some arbitrariness is required in the choice of our parameters, and only orders of magnitude can be obtained. The main points are as follows:

1. The cell surface is endowed with many asperities of various shape that were given different names such as microvilli, lamellipodia, blebs, veils, and ruffles. A typical surface asperity is a microvillus of about 0.1 μm thickness, corresponding to a protrusion of the plasma membrane surrounding a bundle of microfilaments (see, e.g., Reference 27 and Chapter 8).
2. The surface density of membrane-intrinsic proteins was taken as $10^4/\mu m^2$, and these were modeled as spheres of 50 Å diameter. Hence, the mean distance between these molecules on a plane section of the membrane is about 200 Å. This estimate is fairly consistent with the size of intercalated particles and the dimension of protein domains.
3. The glycocalyx was modeled as an assembly of polysaccharide chains with an extremity bound to the cell surface and a length of about 200 Å, which is somewhat intermediate between the length of side chains of intrinsic glycoproteins (i.e., about 5 residues or 25 Å) and a proteoglycan lateral chain of 100 residues (i.e., about 500 Å). Admittedly, this is only an order-of-magnitude estimate. The molecular density in the glycocalyx

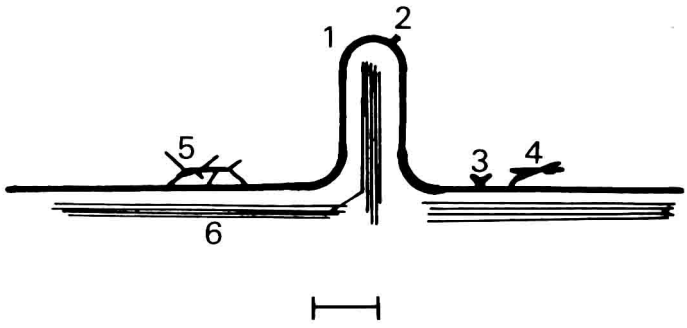

FIGURE 2. Cell surface asperities (10 nm level) with emphasis on the relative size of cell surface asperities. (1) Microvillus, (2) class I histocompatibility molecule, (3) surface immunoglobulin, (4) fibronectin, (5) proteoglycan, (6) microfilaments. Bar is 100 nm.

region may be estimated by taking as a typical membrane composition 10% carbohydrate and 45% lipid,[43] and assuming that lipids account for all the membrane area (the contribution of proteins to the cell-surface area depends on their state of aggregation, but this is not likely to alter our estimate by a factor lower than 1/2). Assuming an area of 70 $Å^2$ per phospholipid and 38 $Å^2$ per cholesterol molecule with a molar ratio of 1 between these species, it is readily found that there is a mean of 2.8 hexasaccharide residues per 108 $Å^3$. The mean density of saccharide residues within the glycocalyx is therefore 1.29 10^{-4} molecule/$Å^3$ or 0.2 *M*. Admittedly, the assumption of constant density within the glycocalyx is a crude approximation. However, we lack sufficient experimental data to propose an empirical law for the decrease of molecular density with respect to the distance to the bilayer.

4. The cell surface density of electric charges is likely to play a role in cell adhesion.[44,45] This charge was often studied with cell electrophoresis.[46] A problem with this technique is that the viscous drag due to the glycocalyx may drastically decrease the contribution to the measured charge of the molecules that are deeply buried in the cell coat.[47,48] This makes desirable a confirmation of experimental results with different techniques. Thus, Eylar and colleagues[49] studied the electrophoretic mobility of erythrocytes from different species and they assayed their sialic acid content with biochemical techniques. The mobility of human red cells was essentially abolished by enzymatic removal of negatively charged sialic acid molecules, and the surface density of these groups was estimated at 1/1369 $Å^2$. Biochemical assays revealed one neuraminidase-sensitive sialic acid group per 679 $Å^2$. The latter estimate leads to a charge density of 1.2 $\times$ 10^6 C/m^3. According to the above estimate for cell-surface carbohydrate density, about 6% of monosaccharide residues would bear a negative charge.

The above estimates are summarized in Figure 2. They will be used to calculate the order of magnitude of different interactions likely to occur on cell surfaces.

Now we shall review some basic results on interatomic or intermolecular forces in vacuum or in aqueous ionic solutions.

III. FORCES BETWEEN ATOMS AND SMALL MOLECULES

A comprehensive coverage of the field of intermolecular forces is clearly out of the scope of this book (and, indeed, many books would be required to do justice to this topic). Reference 50 may be of interest for a short historical perspective. A comprehensive review of older work may be found in the celebrated treatise by Hirschefelder et al.[51] that appeared in 1954.