Chapter 1
Selective Biorecognition on Polymer Surfaces: General Issues

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1.1 Biorecognition: Few Concepts

Biorecognition, or molecular recognition, can be defined as the process in which biological molecules interact. This process is the basis of all biological interactions and therefore a key to sustain living systems. In spite of the paramount importance of these processes, the answers of many questions are still unresolved, as illustrated by Wilchek et al. [1]. For instance, how proteins can recognize other proteins, how receptors recognize specific ligands, or how antibodies recognize antigens has been the center of multiple studies but some of the molecular mechanisms of those interactions are not fully clear yet. The large amount of work developed in understanding biorecognition processes have been realized from different points of view. While several groups attempted to focus on protein–protein or protein–ligand interactions from a biophysical and structural perspective, others focused on complex interaction networks involved in signal transduction pathways both in vivo and in vitro. Equally, protein–nucleic acid, protein–carbohydrate, protein–lipid, and even protein–solvent interactions have been investigated extensively. In spite of the multiple aspects that need further research, several aspects are currently understood and will be briefly described within this section.

Two key aspects of the biomolecular interactions are the binding *affinity* and the *specificity*. On the one hand, the *affinity* defines how tight is the interaction between two specific biological molecules and is characterized by a particular binding energy. The latter can be calculated by the combination of all the forces that contribute

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Table 1.1 Range of binding affinities in biomolecular interactions

Biomolecular interaction	Interaction type	Binding affinity (Kd) (M)
Avidin–biotin	Protein–small molecule	10^{-15}
Antibody–Antigen	Protein–epitope (peptides, sugars, phospholipids, small molecules)	$<10^{-7}$
Aptamer–cocaine	DNA–small molecule	10^{-6}
DNA–DNA	DNA–DNA	10^{-7} (10 base pairs)
Glucose–concanavalin A	Protein–sugar	10^{-3}
Integrins–RGD and GFOGER sequences	Protein–peptide	10^{-5}–10^{-3}

to a defined interaction. Biomolecular interactions present a wide range of binding affinities. The binding affinity constant (Kd) inversely related to the affinity can range from fM-pM in the systems with higher binding affinity such as enzyme–substrate complexes, biotin–avidin system to mM affinities in weak interactions usually related with transient complexes. Table 1.1 shows representative examples of the wide range of binding affinities in natural systems.

On the other hand, the binding *specificity* can be defined as the selectivity of one molecule for a ligand in preference to related ligand molecules. Binding specificity is fundamental for the maintenance of the balance of interactions within living systems. For instance, the cellular milieu is a complex media where many molecules are present; therefore, the binding specificity of the different elements is critical for the balance of the molecular interactions networks that preserve the cell homeostasis.

In a complex cell system, both the binding affinity and the binding specificity, together with the concentration of the different components of a simple interaction network, play interrelated roles that affect the final outcome.

For example, molecule A can bind in the same binding site either molecule X or molecule Y with a 100 times higher affinity for the molecule X (Kd 1 μM) (Fig. 1.1). One can encounter different situations just by changing the concentration of the two ligand molecules in the environment in which: (1) the A molecules only bind molecule at equal concentrations of X and Y X; (2) the A molecules bind equally X and Y when the concentration of Y is 100 times the concentration of X; (3) the A molecule binds only molecule Y, even when the molecule A is more specific for X molecule if there is large excess of the molecule Y compared to X. This example illustrates the different regulation levels to modulate the complex biorecognition interactions. Additional complexity might be incorporated by allosteric effects in which the binding of one ligand to a site results in a conformational change in the target molecule that modulates its affinity for a second ligand molecule.

In addition to the two important considerations described above, the understanding of biorecognition interactions requires the evaluation of the nature of the interactions. In effect, biorecognition is based on a variety of non-covalent interactions and on the structure of the biomolecules. Non-covalent interactions can include H-bonds, dipolar interactions, electrostatic interactions, hydrophobic interactions,

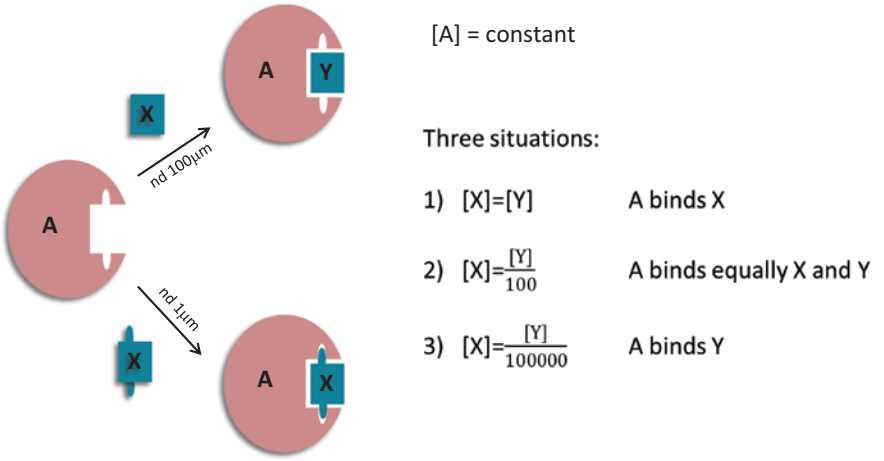

Fig. 1.1 Biorecognition binding affinity and specificity. Molecule *A* binds two ligands *X* and *Y* with different affinities: Kd of 1 μM for molecule *X* and 100 μM for molecule *Y*. Even though the molecule *A* has significantly tighter affinity for molecule *X*, the fraction of *A* molecules bound to *X* or *Y* can change completely depending on the relative concentrations of the ligand molecules

van der Waals forces, cation–π interactions, and π–π stacking. Moreover, usually the biorecognition processes are driven by a combination of a large number of different weak interactions between two molecules. Those interactions are therefore complex to predict, even if the energetics of the individual components is known. In addition to non-covalent interactions, shape complementarity has been evidenced to also take part in biorecognition processes [2].

Biorecognition interactions can be on one hand transient and very dynamic; usually these interactions are involved in regulatory processes in cells. On the other hand, these interactions can be very tight such as the interactions that hold the two strands of the DNA double helix together, even though it can also be opened by proteins such as helicases that break the H-bonds between the DNA strands. In general, biomolecular interactions are regulated by a subtle balance between many interactions.

The interactions exhibited between two biomolecules can also be defined as *dynamic or static* (Fig. 1.2). In *static interactions* the two molecules bind without a conformational change of any of the components. In this type of interactions the recognition site is shaped to specifically recognize a molecule, or molecules. *On the contrary, dynamic interactions* are more complicated and can comprise a variety of recognition modes. The common aspect is that the molecular recognition includes a conformation change. This can be a simple rearrangement of a binding pocket to accommodate the ligand or can be a more complex allosteric effect in which the binding of the first ligand to one binding site induces a structural change that affects the association of a second ligand to the same or another binding site. These allosteric effects can be positive, negative, double, triple, and include all the range of potential combinations.

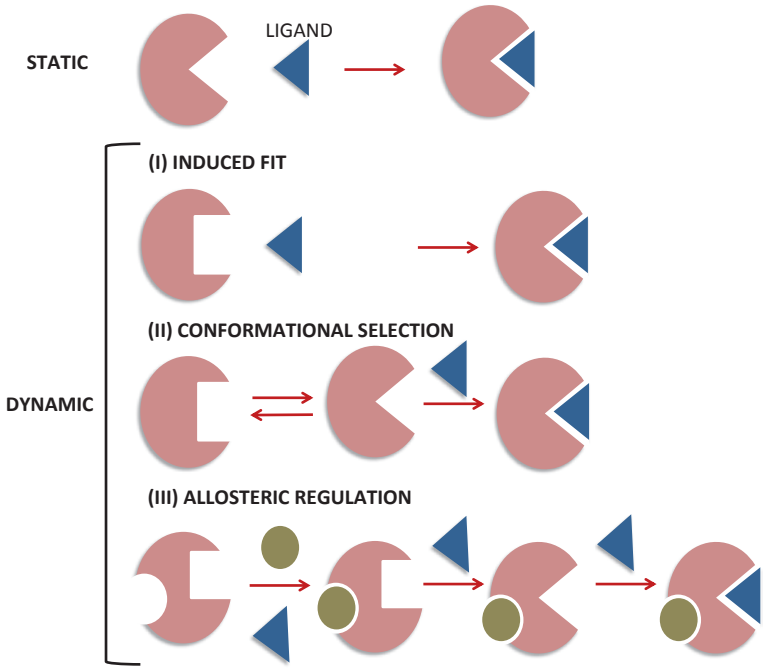

Fig. 1.2 Schematic representation of static and dynamic recognition mechanisms

Here we want to present the fact that biomolecular recognition can be as simple as the recognition between two base pairs, which occurs in the DNA to a very complicated system that can lead to complex molecular machines made of multiple components such as the bacterial flagellar motor [3]. Nowadays and thanks to many years of research in the field of biomolecular interactions, studying the biophysics and the structural basis of countless interactions, we have started to unravel the great complexity of biorecognition.

1.2 Biorecognition Plays a Key Role in Living Systems

The complexity of biorecognition as mentioned before is all encoded in the natural living systems. Biorecognition is mediated for an array of different biomolecules, including nucleic acids, proteins, sugars, lipids, and small biomolecules. Therefore, all the cellular functions rely on biorecognition events. More over on the balance of many biorecognition events that take place at the same time in the intracellular environment and are all interrelated in a delicate equilibrium.

For example during the protein biosynthesis inside cells, the different amino acids are linked in a sequence to form protein chains that fold with a define structure that will encode a specific function. This complex process is carried out by the

Fig. 1.3 *C. elegans* interactome map, showing 5500 protein interactions among 3000 proteins. Each *dot* represents a protein and each *line* between them represents a protein–protein interaction [6]

ribosome, a large biomolecule inside the cells composed at the same time by many ribosomal proteins and ribosomal nucleic acid (rRNA). The ribosomes recognize the messenger RNA sequence and translate it into a protein sequence. For this translation process the ribosome has to recognize in a concerted way many different molecules including coding mRNA, aminocyl tRNA molecules that carry the different amino acids, ATP molecules that provide energy, and different regulatory proteins such as initiation factors [4, 5].

Biorecognition processes vary in complexity and thus the investigation of such processes can be difficult. For instance, to illustrate this complexity we show a map of the protein–protein interaction network, or interactome of a simple multicellular organism, the worm *Caenorhabditis elegans* (Fig. 1.3). The figure shows the large number of potential interactions and the multiple interaction partners for each protein. In addition to the evident complexity of those biological networks of interactions, there is an extra level of complexity encoded by different binding affinities and specificities and the dynamics of those interactions.

The complexity of all biomolecular interactions in a single cell, considering not only proteins but also nucleic acids, sugars, lipids, and small molecules, is enormous, and the failure of one of these interactions might cause severe diseases, even death.

1.3 Application of Biorecognition to Synthetic Systems

Due to the large array of biorecognition interactions in vivo, we can take advantage of a broad range of to generate synthetic complex systems with defined properties. More precisely, natural biorecognition interactions can be selected based on the specific needs in terms of affinity, specificity, modularity, and dynamics of the interaction needed. In addition, different biomolecules also give a selection window for experimental conditions in terms of temperature, pH, and osmolarity in

which the selected interaction is stable. Finally, the key features of the biomolecular interactions can be modulated by design to generate biorecognition systems with optimized properties for particular applications [7–12].

The development of synthetic components with molecular recognition capabilities is a current center of interest for many different targets. As will be depicted throughout this book, biomolecular scaffolds with molecular recognition elements, i.e., those employed in synthetic approaches, can be classified in four different types: proteins and peptides, nucleic acids, small organic molecules, and synthetic polymers [13]. Special scientific attention is currently being paid to these systems, and the technology of producing and improving novel molecular recognition elements is rapidly evolving [14–17]. As a result, today, specific biorecognition molecules have been adapted in different synthetic devices for different applications, including diagnostic testing and biosensing. In addition, such systems have been employed for therapeutic purposes by assisting drug delivery or modulating genetic expression [18–20].

1.4 Biorecognition at Surfaces

The biorecognition in biological systems is mostly in solution, but within the living systems there are many key recognition events that take place on immobilized systems such as the 2D biological membranes. The particular effects on biorecognition of immobilized systems need to be considered for the successful applications of those systems. For example, one key problem of biorecognition on surfaces is the establishment of efficient mass transport between the bulk solution with the ligand and the surface with the recognition moiety. If the mass transfer is not efficient, a concentration gradient of the ligand will be generated in the solution. This effect needs to be considered for each system and will depend on the interaction affinity, the number of binding sites on the surface, and the concentration of the ligand in solution [21, 22].

Another limitation that would apply only for kinetic studies is the deviation from first order biding kinetics observed in the surface binding processes. These deviations might be due to the heterogeneity of the immobilized binding sites and their orientation on the surface [23]. The density of binding sites immobilized on the surfaces is also critical and should be considered when using biorecognition on surfaces. High density of the ligands might induce avidity effects when multivalent ligands are used. The avidity of a multivalent interaction is the accumulated affinity of different interactions, and can be considered as a functional affinity. Avidity can be a problem to avoid, or an advantage to be used to convert a low affinity interaction into a high avidity one.

1.5 About this Book

Within this context, this book aims to provide a general overview of the strategies that can be employed to prepare micro- and nanostructured polymeric substrates with biorecognition capabilities. In addition, we illustrate biorecognition processes occurring on surfaces, their particular features, and their potential applications.

Chapter 2 establishes the criteria from the point of view of the design of a particular platform introducing the major strategies that are discussed further in detail throughout the different chapters.

Chapter 3 focuses on the description of the molecules that can be incorporated into the platforms, and the basis of the distinct molecular biorecognition processes, including the development of affinity and specificity. This chapter establishes the basis for a knowledge-based selection of biorecognition molecules tailored for the different applications.

Chapter 4 describes the chemical approaches that can be followed to anchor biomolecules to polymeric surfaces. In effect, chemical and physical approaches to modify polymeric surfaces to immobilize biomolecules are thoroughly described.

The following chapters are devoted to the fabrication of different structures and simultaneously biofunctionalization of such polymer surfaces in view of their use in biorecognition processes. In this concern, Chap. 5 introduces the preparation of gradient polymeric surfaces. In these surfaces, a particular variable varies gradually from one extreme to the opposite of the material. Micro/nanoscale structuration, chemical functionality or surface mechanical properties are aspects that will be discussed in detail. Finally, this chapter describes how these materials can be fabricated in view of use in applications such as cell sensing.

Chapters 6 and 7 explore two closely related strategies to pattern polymer surfaces with micro- and nanometer scale resolution. On the one hand, Chap. 6 focuses on the description of the polymer replication techniques in which a master stamp is brought into contact with the polymer; as a result of the pressure applied, the polymer adopts the form imposed by the stamp. On the other hand, Chap. 7 describes the lithographic approaches including conventional lithography and nonconventional (soft lithography or printing processes) lithography, electron beam lithography, and focused ion beam that allows the preparation of surfaces with topographies at the nanoscale.

Inkjet printing that enables the precise deposition of very small droplets of liquids in a well-defined and user-controlled position of a particular substrate is depicted in Chap. 8. More precisely, in this chapter, the preparation of biorecognition surfaces by immobilization of DNAs, enzymes, or antibodies of various polymeric surfaces with high precision is analyzed.

Porous interfaces with controlled pore distribution and size have also been employed for biorecognition purposes. In Chap. 9, a particular approach to prepare porous surfaces with pores sizes ranging between 20 nm and 20 μm is described. The approach employed, known as Breath Figures, also permits the control over the

chemical composition of the pores and thus the precise immobilization of biomolecules with biorecognition capabilities.

Chapter 10 describes the preparation of polymer brushes, i.e., the immobilization (covalent or not) of polymer chains onto surfaces. Polymer brushes can be designed to include different biorecogntion sites such as RGD or GFOGER sequences that enable the protein recognition. Moreover, cell adhesion and immobilization of proteins is reviewed.

Using microfluidic systems with functional patterned surfaces is also an interesting strategy to construct polymeric platforms with final recognition capabilities. Microfluidic devices have great potential to be widely used as a great diagnostic technique of many diseases, especially in remote areas where well-equipped biochemical labs and trained technicians are generally not available. A detailed description of microfluidic systems is provided in Chap. 11.

Colloidal structures arranged on polymeric surfaces can serve to create different surface patterns. In Chap. 12, apart from the description of the fundamentals of formation of colloidal templates, a large number of the patterns analyzed involve biomolecules that can be precisely distributed.

Chapters 13 and 14 are devoted to sophisticated systems to obtain biorecognition platforms. Chapter 13 resorts to the preparation of a 3D polymeric structure prepared by using a target analyte acting as template, i.e., the so-called molecular imprinting. Upon copolymerization of the monomer mixture, the removal of the analyte provides binding sites which are complementary in size and shape to the analyte and thus allow their rebinding. This strategy, in comparison with other approaches, provides not only the selectivity due to the chemical functionality but also shape selectivity. Chapter 14 focuses on the tip-based strategies to finely control the deposition of biomolecules using an atomic force microscopy (AFM) tip. This nanofabrication approach, also known as tip-based nanofabrication, is described in detail in three variations: dip-pen nanolithography, nanoshaving, and nanografting.

Finally, Chapter 15 presents an overview of the major issues that remain unresolved as well as the future trends for the development of more performant polymer surfaces with biorecognition capabilities. In particular, biocompatibility of the interfaces, stability as well as binding specificity are current challenges that require further investigation.

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