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TOPICAL REVIEW

Bio-specific recognition and applications: from molecular to colloidal scales

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Abstract

Biomolecules have the well-known ability to build reversible complexes. Indeed, antigens and antibodies or adhesion molecules are able to recognize one another with a strong affinity and a very high specificity. This paper first reviews the various techniques and related results about binding and unbinding, at the scale of a unique ligand/receptor couple. One important biotechnological application arising from these recognition phenomena concerns immunodiagnosis, which is essentially based on the formation of these specific complexes. We show how the physics of colloids associated with the growing scientific background concerning molecular recognition helps in rationalizing and inventing new diagnostic strategies. Finally the concept of colloidal self-assembling systems as biosensors is presented as directly impacting the most important questions related to molecular recognition and their biotechnological implications.

Contents

1. Introduction

A biosensor is aimed at determining various parameters associated with biological species such as single biomolecules, molecular complexes, viruses, cells and tissues. The understanding of mechanisms, from a molecular scale up to the cell functions is certainly one of the most important scientific issues concerning molecular biology. It simultaneously requires

information dealing with concentrations, structures, conformations and dynamics, both at different length and timescales. The possibility of measuring such parameters requires the development of as much as possible sensitive, selective, real-time and multi-scale biosensors.

One crucial aspect of biomolecules concerns their ability to build reversible complexes through specific molecular recognition mechanisms. Indeed, a major property of biomolecules is to bind a variety of ligands in order to fulfil a specific function such as mediating cell adhesion, triggering receptor-mediated cell activation or regulating intracellular networks and immuno-responses. Probing recognition phenomena has given rise to various types of sensors focusing on rupturing or binding, single as opposed to multi-molecular events which concern adhesion of mesoscopic objects (vesicles, cells or drops), and finally thermodynamic or kinetic aspects of bulk ligand–receptor reactions.

One important biotechnological application arising from these recognition phenomena concerns immuno-diagnosis, which is essentially based on the formation of these specific complexes. We show how the physics of colloids associated with the growing scientific background concerning molecular recognition helps in rationalizing and inventing new diagnostic strategies. Finally the concept of colloidal assembling as biosensors is presented as directly impacting the most important questions related to molecular recognition and their biotechnological implications.

2. Probing molecular recognition

Classical biochemistry methods give the thermodynamic and kinetic constants for ligands and receptors which are both in solution, or when one of them is being immobilized onto a surface [1]. These constants can be readily determined with new tools, measuring the variation of evanescent wave intensity upon binding of antigens on an antibody-coated surface [2]. However, for a lot of relevant situations, biomolecules are attached to the cell surface, so their fluctuations become strongly restricted, as a result of anchoring, crowding from neighbours and eventually compression due to adjacent cell surface proximity. An important challenge in biophysics concerns the characterization of these effects, which certainly requires the achievement of new tools.

The more mature aspect concerns the study of single bond rupturing under mechanical force. A typical bond lifetime, measured in solution, varies from 1 s to 10 min for classical antigen–antibody couples, and up to several hours for the well-known streptavidin–biotin couple, which is the strongest non-covalent link encountered in biology. Using AFM [3], and later using red cell sensors and vesicles, both handled by micropipettes, rupturing of a single molecular complex has been precisely studied [4]. Thermal activation is a critical aspect of bond failure: indeed, specific links will fail under any level of force if held for a sufficient amount of time. Starting from a lifetime of 55 h when the two partners are in solution (no external force), the lifetime of biotin–streptavidin will drop to 1 min or so under a 5 pN applied force, and to 3 ms for a force of 200 pN [4]. Not only the level of the pulling force, but the time of application as well, or the loading rate of that force, is an important parameter controlling the bond failure. More precisely, the so-called dynamic force spectroscopy probes the energy landscape of a single molecular bond along the force-driven pathway. It was shown that the separation of a ligand from its receptor under force followed a one-dimensional path, with an energy–distance curve determined by the so-called energy landscape, displaying sequential maxima or barriers. The plot of unbinding force versus the logarithm of the loading rate is found to be composed of sequential straight segments, the slope of which might be written as kT/x_i, where x_i represents the distance between the location of the energy minimum and the ith barrier on the energy landscape [5]. From an experimental point of view, working on

single molecules implies several hundreds of attempts to obtain a reliable statistic regarding its stochastic nature. Nevertheless, it should be noticed that, although Bell proposed the underlying ideas 25 years ago [6], measurements became possible in the mid-1990s only. Until very recently, all the observations have shown the intuitive fact that force shortens the lifetime of the bond ('slip bond'), but new experiments demonstrate that the lifetime of P-selectin to its ligand can increase with the applied force [7] ('catch bond')! The mechanical constraint may induce conformational changes such that the molecules are locked more tightly [8, 9]. Today, the single molecular character of these dissociation measurements is without doubt well accepted [10].

The case of binding is quite a bit more complex. From a theoretical point of view, all the non-specific forces on the surfaces must be taken into account [11] and there is no longer a unique one-dimensional path imposed by the external force: the energy landscape can be significantly more complex. For instance hydrodynamic interactions and thermal fluctuations of surfaces may have subtle consequences and, as an additional complexity since the surfaces are not pulled apart, rupture events and rebinding can superimpose. From an experimental point of view, the general principle of the various types of measurements performed on the binding rate at a single complex scale is always the same: first, the surfaces are brought into contact or at a particular distance and eventually by also controlling the velocity of that motion. Second, the molecules are left for a controlled amount of time to react and third, the surfaces are pulled apart to determine whether or not an association has occurred.

The atomic force microscopy (AFM) probes the binding of ligands and receptors by grafting or adsorbing one of them onto the tip and moving that tip towards a grafted surface. The contact duration and the loading force at contact can be changed. In turn, the frequency of binding and the outwards force profile which results from the binding process until rupture occurs can be measured. In other words, the binding scenario that takes place under given conditions is depicted through the rupturing signature at a given pulling velocity. Following this strategy, fibrinogen was shown to adhere in a multimeric configuration once a minimum contact duration is allowed as required for exploring these multimeric modes which confer a much greater adhesive strength [12]. Binding of P-selectin to P-selectin glycoprotein ligand-1 has also been explored with AFM [13]. Interestingly, the adhesion probability in the P-selectin/PSGL-1 system increases with faster pulling velocities. This phenomenon may be a general feature of selectin/ligand interactions and seems to be the underlying molecular property of the increase in the leukocyte tethering probability with increased shear flow observed in L-selectin rolling experiments [14]. Cadherin-mediated intercellular adhesion is a basic cellular function involved in a variety of physiological and pathological processes, including embryogenesis, epithelial barrier regulation and tumour cell metastasis [15]. Therefore the calcium-dependent homophilic adhesion between cadherin was also carefully examined with AFM [16]. A consolidation of the unbinding strength is clearly shown as a function of the contact duration (from 0.1 to 0.5 s), underlying the multimerization of tip and plate bound cadherins.

With some improvement, the biomembrane technique allows measurements of the binding kinetics: the idea is to probe the dependence of adhesion probability on contact duration and the densities of ligands and receptors [17]. It has been shown that the same ligand–receptor couple placed on different types of cells will present the same reverse kinetic rate, but very different forward rates. This was attributed to the cell's micro-topology, which was different for a smooth red blood cell and a Chinese hamster ovary cell. The latter presented microvilli tips [18], so for the same apparent surface contact, the actual contact area was very different. Among the few published results, the experiments show good correlation between function and binding rate: for example, the forward rate of the E-selectin/carbohydrate ligand interaction,

which initiates the multi-step adhesion in the recruitment of leukocytes from the circulation to inflamed tissues, is three or four orders of magnitude higher than that of Fcγ receptors to their IgG ligand [19]. Up to now, the existing theory [1] failed to relate the so-called 2D kinetic rates between surface-bound molecules and the 3D kinetics rates, where one or both species are in solution [20].

The laminar flow chamber operated at low shear rate can provide additional information on the fine properties of ligand–receptor association and rupture [21]. Binding frequencies are obtained by pooling all colloidal trajectories in a given experiment and calculating the ratio of the number of steps followed by an arrest, divided by the total number of steps. In the same way, from the statistics of arrest durations, the unbinding frequency is computed. This technique gives valuable information about the role of shear rate as compared to shear stress [22]. In the case of neutrophils cells interacting with P-selectin, by varying the viscosity the authors showed that tether formation is related to shear rate rather than to shear stress. This technique can also demonstrate the existence of transient states during the process of binding, as in the case of streptavidin-coated microbeads interacting with a biotin-derivatized surface [23].

The surface force apparatus (SFA) is also a means of probing some collective binding processes on model surfaces. First, due to the very precise distance measurement, it has been shown that the attractive force due to specific recognition is very short range in most cases: less than 1 nm [24]. Second, due to the macroscopic dimensions of the surfaces, and therefore the large number of bonds involved within the contact (more than 10 000), the force–distance profile appears simply as the superposition of the non-specific forces and the short range protein–ligand attractive well [25]. Lastly, the measurement of the macroscopic adhesion force as a function of surface distance, after a reaction time of about 1 s, allows the rate of binding to be inferred. It was successfully attributed to the molecular spacer dynamics when dealing with the biotin–streptavidin couple, when one of them was held by a linear short chain spacer [26].

In the case of soft surfaces, after the first recognition event, consolidation of adhesion is strongly coupled to the deformation of interfaces. Reflectance interference contrast microscopy (RICM) follows the formation of specific adhesive patches between a model grafted solid surface and a vesicle or a cell; the role of repelling macromolecules coupled to the presence of specific linkers, the importance of linker diffusion on the surface and the growth kinetics of the adhesion patches have all been addressed in some detail [27].

3. Biotechnological implications

Recognition has also many biotechnological implications. A major one concerns biological diagnosis or sorting of biomolecules, viruses and cells. In most cases, a targeted species is probed using a complementary molecule, which forms a complex, such as a ligand and a receptor, an antigen with an antibody, or two complementary nucleic acid strands. The challenging aspect of diagnostic technologies is to process the molecular scale recognition step resulting from the formation of as few a number of pairs as possible into a more macroscopic signal and from within a biological mixture containing many different species. Today, developing simple, low cost, fast and highly sensitive procedures to measure the concentration of biomolecules within serum, plasma, or under certain environmental conditions is certainly one of the major challenges of life sciences.

Immunoassays are based on antigen–antibody recognition. The antigen is so-called because it induces an immune response. The immune response is a process through which the antigenic species is recognized as a foreign species and further deactivated: the capacity to

yield diverse antibodies with unique specificities is enormous [28]. Early immunochemistry was based on the precipitation of large complexes made of antibodies and antigens. Following the same process, the use of Brownian particles, which quickly find their target, significantly improved detection sensitivity because of the increase in scattered light when aggregation between grafted colloids takes place. As the simplest but very general example, let us consider an antigen having two different epitopes for two antibodies A and B. To reveal the presence of such an antigen, particles grafted with A and B antibodies and the sample are mixed. The formation of small clusters is then expected, the rate of which depends on many factors. Change in light scattering due to the presence of these small clusters will reveal the existence of sandwich-like structures: A–Ag–B (latex agglutination immunoassay). These homogeneous assays, as opposed to heterogeneous assays in which washing steps are necessary prior to detection, are today by far the most simple and straightforward: they were introduced more than 40 years ago [29] and today several hundreds of different tests based on this principle can be found on the market, mainly for the detection of infectious diseases and protein quantification [30].

Regarding heterogeneous assays, the most extensively used is the ELISA assay (enzyme-linked immunosorbent assay). Magnetic particles are very useful for ELISA because magnetic separation is rapid and convenient. In that case, antigen capture is achieved through the recognition of only type A antibodies, which are grafted onto the magnetic particles; note that there is no possible colloidal aggregation in that case because no sandwich-like structures are possible at that stage. After washing, labelled B antibodies are added to form the sandwich structure; the label or the so-called tag of these second antibodies may be either an enzyme (ELISA) or a fluorescent, radioactive or chemiluminescent molecule. After incubation, the sample is washed again in order to carefully remove the excess of labelled antibodies, the signal then being detected. Because of these two washing steps, a sensitivity as low as $\mathrm{pmol\,l^{-1}}$ is routinely obtained in automatic systems. The main drawback of this approach concerns its complexity and therefore its associated cost as well as the time required compared to homogeneous assays. It should be noticed that the ELISA method, which was introduced in the 1970s [31], is used in all commercial instruments once high sensibility is required, with test durations varying from 1 to 2 h.

The challenge today for physicists, chemists and immuno-chemists is therefore to make homogeneous tests much more sensitive. Today, most of the ongoing research on agglutination tests aims at improving their limited sensitivity ($\sim\mathrm{nmol\,l^{-1}}$). In order to do so a detailed understanding of the different steps leading to specific sandwich structures when starting with Brownian grafted colloids should be addressed. Therefore this more applied question is readily linked to the more fundamental aspect of probing single molecular complex binding, as previously discussed.

Concerning the agglutination assay the more stringent limitation is related to colloidal stability in such complex biological media, characterized by a very high ionic strength and containing a lot of very adherent proteins such as fibrinogen, which is found in plasma [32]. It is clear that aggregation must only occur in the presence of antigens, and never be due to any attractive colloidal forces (van der Waals, hydrophobic or electrostatic) or to adherent proteins. Therefore, some kind of repulsive forces must exist between particles to ensure the absence of non-specific links, or so-called 'false positives'. However, since antigen–antibody recognition involves quasi-contact forces (<1 nm, see [33]), increasing colloidal stability systematically decreases immuno-reactivity. Chemical and biochemical know-how has been developed [34] about the core and surface of particles (being responsible for colloidal stability and the possibility of having functional groups for further grafting with probes or ligands), about the antibody fixation (type and density, adsorption or covalent coupling, saturation with

other proteins, etc) and about the medium which affects the optimization of the test (pH, ionic strength, surfactants, depleting agents, etc). It turns out that all parameters are strongly coupled together and therefore conditions must be redefined for any change of antibodies, antigens or particles [35]. However, with this simple strategy, there is always some non-specific aggregation, which is superimposed on a specific one, imposing the limit of the sensitivity. Indeed, the measurable level of antigen in solution that can be detected can never exceed roughly the number of non-specific links that form between beads during the test. Moreover, this number is strongly related to the nature of the surrounding fluid, so the variability of biological fluids limits even more the degree of reliability of the detection in the lower concentration limit.

The second limitation is related to the detection of aggregates. Since we are interested in determining low concentrations of antigens, the problem is essentially shifted to quantification of the doublets' concentration. To do so, the more widely used technique is turbidimetry, because of its simplicity. As long as beads are small compared to the wavelength of the employed light (which is not exactly the case in practice), a doublet will scatter twice as much light as two separated beads would do. In the low antigen concentration regime, which means that the bead concentration is larger than the antigen concentration, the scattering from singlets (non-aggregated beads) will add to the signal originating from the doublets. In contrast to ELISA-like assays, where excess secondary labelled antibodies are washed out prior to detection, the homogeneous assay is performed still keeping the full matrix. Therefore, the number of doublets that can be detected is ultimately limited by the total number of beads in solution. One possible solution would be to simply decrease the particle volume fraction ϕ in order to increase the sensitivity. However, lowering ϕ directly affects the kinetics of doublet formation, which may eventually become too slow. If we assume an ideal system where the immune recognition is diffusion-limited, the average time t_b for a doublet to form is [36]

$$t_b = a^2/(6D\phi)$$

where a is the particle radius, ϕ is the volume fraction and D is the diffusion coefficient. We find t_b of about 1 s when typical values for a and ϕ are, respectively, 100 nm and 0.1%. In fact, the specific link formation is reaction-limited, owing to the intrinsic antibody–antigen association rate [1] or due to the repulsive colloidal forces, as previously considered. In this regime, several collisions are necessary for a specific link to form. Therefore dilution will dramatically increase the assay duration, which precludes any optimization in that direction.

Several strategies have been proposed to overcome the noise caused by non-specific links. Single molecule studies are certainly inspiring these strategies. They are based on force differentiation, originating from the fact that non-specific links remain weaker than specific ones. Let us give an example of this strategy: first, an immunochemical sandwich is expected between a grafted wall and a magnetic bead. Second, a magnetic field is applied in a controlled manner so that the applied force on particles is enough to break up the non-specific links only. The remaining beads are then counted with a CCD camera [37], using a magneto-resistive transducer [38] or a CMOS Hall sensor [39]. Recently, this idea of force differentiation was extended, using a DNA zipper as a local force probe able to contrast specific and non-specific bonds [40]. The authors claim better or comparable results than ELISA-like assays for these examples, but experiments are not always done in a real complex matrix. It should be noticed that the first washing step in an ELISA assay may be considered as helping to remove part of the non-specific capture as well and also participates in a force differentiation strategy.

To tackle the immuno-reactivity/colloidal stability paradigm, a strategy using ultrasonic waves has been proposed [41]. It is still a classical agglutination format, but during the incubation time between beads and antigens the sample is placed within an ultrasonic apparatus.

Sensitivity is increased 10–1000 times, depending on the nature of the antigens, particles size and type. Two phenomena may be responsible for this enhancement. An ultrasonic standing wavefield subjects the particles to a radiation force and drives them towards pressure nodal regions. First, the local concentration of beads increases, therefore increasing the collision probability. Second, the applied force can be enough (of the order of 1 pN [42]) to overcome the repulsive colloidal force barrier.

Finally, several strategies have been proposed to improve the detection: they share the same underlying idea, lowering as much as possible the background signal due to non-aggregated particles. Colloids can be synthesized with a high level of refinement including controlled size, surface and content, which allows us to implement new adjustable properties. Tiny gold particles will change colour upon specific agglutination due to a shift in the plasma resonance [43]. Furthermore, when coupled to temperature changes (melting studies), such shifts can reveal single base pair mismatches between complementary DNA strands [44]. In the luminescent oxygen channelling immunoassay (LOCI [45]), 'donor' and 'acceptor' beads are used to form the sandwich: the donor particle produces excited singlet oxygen under 680 nm light excitation. This oxygen diffuses across and initiates a chemiluminescent reaction in the vicinity of the acceptor bead. The selectivity and sensitivity of this method originates from the short diffusion distance of the oxygen singlet ($<$100 nm). Another strategy consists in tracking aggregates or particles one by one. In the Copalis^{TM} technique [46], as in flow cytometry, a fluidic system aligns particles in a single line. They pass through a laser beam, and from their distinct scattering patterns it is possible to count the number of singlets, doublets, etc. Related techniques are based on the formation of a sandwich-type structure between a grafted bead and a secondary fluorescent antibody, which recognizes the desired antigen as in ELISA. However, beads may be differentially tagged, allowing multiplexed detection by using a flow cytometer [47]. Two-photon excitations have also been used for the same purpose; here, the sampled volume is so small that there is virtually no background fluorescence [48]. In both methods, the scattering signal originates from the tiny probed volume while the particles act as local concentrators of antigens, increasing the assay performance by orders of magnitude in terms of concentrations [49]. Another detection method uses a SQUID (superconducting quantum interference device) microscope to measure the relaxation time of the particle's magnetization. The immune sandwich is formed between a grafted wall and the magnetic beads. Particles in solution undergo Brownian rotation, while for the bound particles only Néel relaxation is allowed. Since the timescale of these two relaxations is quite different [50] a precise differentiation is possible.

4. Self-assembling of magnetic colloids: molecular recognition and applications

This part introduces the basics of using superparamagnetic colloids and their spontaneous self-assembling ability under a magnetic field, as new biosensors. The possibility of varying magnetic interactions, and therefore the distance between particles within chain-like structures, addresses new possibilities; first, the ability to resolve recognition at a molecular level and, second, as a new tool for diagnosis. However, the particle must fulfil some very specific requirements in order to be used with this strategy: particles must be spherical, monodisperse, strongly magnetic and Brownian. This last characteristic imposes a diameter of about 500 nm maximum. In addition, their surface must provide very strong colloidal stability in any type of buffer, serum or plasma. Finally the particles must also possess functional groups in order to be further grafted with the biomolecules of interest. Such particles are now commercially available and manufactured by Ademtech [51].

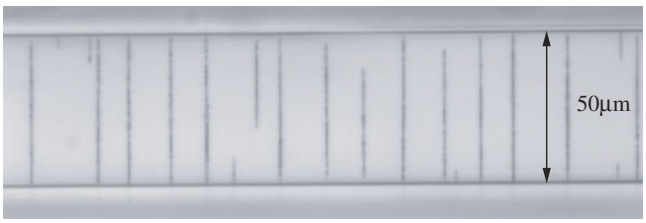

Figure 1. Chains of particles aligned with the magnetic field in a square capillary tube of 50 μm (optical microscopy, $\times$100 objective).

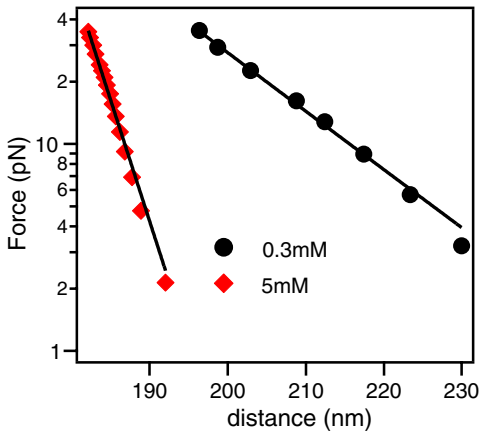

Figure 2. Evolution of the force with the centre-to-centre distance for two ionic strengths. Points correspond to experimental values, the full line to theoretical predictions (the surface potential is the only fitting parameter).

(This figure is in colour only in the electronic version)

The application of a magnetic field induces a magnetic dipole in each bead and they form chains due to Brownian motion coupled to dipolar interactions. These structures are reversible: by switching the external field off, the magnetic moments vanish and the Brownian motion instantaneously disrupts the one-dimensional ordering. At low concentrations, the chains are one particle thick, finely separated and aligned along the field direction, as shown in figure 1. The one-dimensional order of the chains gives rise to a very remarkable interference pattern which is used to measure the inter-particle distance from collecting the Bragg scattered light at 180° [52]. Moreover, knowledge of the magnetic content of each bead and their separation within the chain (deduced from Bragg scattering) allows computation of the applied magnetic force. Since the colloidal repulsive force strictly balances this attractive magnetic force, the force–distance profile is readily determined for various conditions. One example is given in figure 2 where electrostatic forces are characterized as a function of ionic strength. This technique originally offered the first direct measurement of electrostatic repulsion between colloids and the validation of the predicted exponential decay (Debye) [52] and has since been used to characterize various types of colloidal interactions [53–55].

Once the particles are grafted with ligands and put in the presence of receptors able to recognize two of them, the chains are no longer reversible and, instead, specific recognition eventually leads to a sandwich-type molecular construct that can link particles together. The number of links per particle can be tuned by choosing the appropriate concentration of both

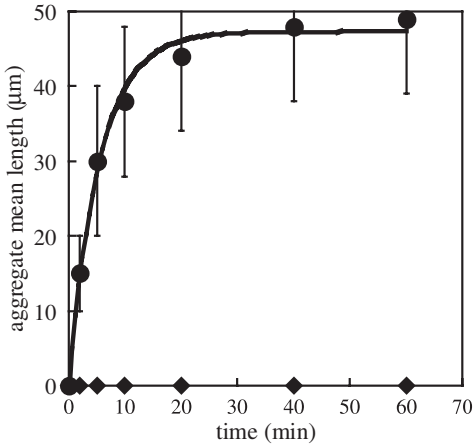

Figure 3. Kinetics of aggregation of streptavidin-coated magnetic particles (volume fraction 0.02%) in the presence of biotinylated BSA (concentration 5 nmol l^{-1}) in a 50 mmol l^{-1} phosphate buffer, under a 9 mT magnetic field (circles), and without a magnetic field (diamonds); the full curve is an exponential fit.

grafted ligands and receptors. In the low concentration regime it is therefore possible to build chains made of single links [56]. Therefore self-assembling allows a control of both force and distance between particles, as well as measuring specific binding kinetics on a single link basis. In our strategy links can form in the presence of a magnetic field only: inhibition of sandwich formation in the absence of a sufficient field results from the existence of a long-range repulsion between magnetic colloids, mostly electrostatic in origin, which can be carefully controlled. This ensures a careful control of the geometry before the reaction starts and precise kinetics measurements.

As a generic example of the underlying aspects of this strategy we show in figure 3 the rate of linking between streptavidin-coated particles when mixed with biotinylated BSA (bovine serum albumin). The experiment is done in a square capillary tube of 50 μm width and the aggregate mean length is determined by optical microscopy as a function of the incubation time under the magnetic field. In the absence of a magnetic field, repulsive colloidal forces are sufficient to totally inhibit the formation of a link. Therefore the colloidal suspension remains unchanged with no detectable aggregation. If the field is applied for a certain time and then switched off, some particles remain bound together within doublets or short chains, depending on the concentration of BSA which acts here as a model of the antigen. Repeating the experiment for different periods of time allows a determination of the binding rate as a function of the magnetic field, which imposes the colloidal distance (see figure 3).

Note that, in this approach, the stochastic character of the recognition is already considered since billions of links are probed, all of them performed in the same average geometrical configuration. In some sense, this experiment is comparable to the one performed with AFM or the biomembrane force probe (see section 2). However, the magnetic chain simultaneously probes a multi-parallel system. The main underlying idea is therefore to apply suitable mechanical and geometrical conditions, within a sufficient time, to induce specific recognition. As emphasized before, in contrast to bond rupturing, very little is known about binding, once biomolecules are grafted onto colloids, cells or drops. It seems that a 'long' contact time, more than an applied force, is efficient [23]. The major goal, in using this new tool, is determining the rules that govern the kinetics of binding, both for specific (recognition) and non-specific (eventually protein-mediated) routes.

In terms of biotechnological application, agglutination assays can be greatly improved using these magnetically induced structures. The basic idea is to exploit chaining to catalyse recognition. The strategy remains very similar to that of classical agglutination tests except for the presence of a magnetic field and the use of grafted magnetic colloids. First, the grafted beads are mixed with the sample to be probed, such that the antigens can be captured. Second, a sufficient magnetic field (about a few tens of milliteslas) is applied to form chains, so that antigens can irreversibly link particles. Third, the field is relaxed and the amount of the remaining chains is detected, reflecting the antigen concentration. Chains, including doublets, triplets and so on, are easily detected with simple turbidimetry, as is usually done in classical agglutination tests. It has been shown that the assay sensitivity can be improved by a factor of 1000 [57]. The origin of this enhancement is still to be determined. It could be closely related to the case of 'ultrasonic agglutination assay': since a force is also applied on the particles, the repulsive colloidal barrier can be overcome. Moreover, particles are also brought into close contact for quite a long time compared to what is expected from Brownian collisions, greatly enhancing the probability and duration of the encounter between grafted macromolecules.

5. Conclusion

The principles underlying molecular recognition, including binding mechanisms and kinetics, besides the more classical thermodynamic aspects, are still to be elucidated. The unbinding regime is much more mature, mainly because experimental complications have been less of a limitation. The many aspects that have been described throughout this review give the flavour of the very rich ensemble of phenomena that are responsible for these multiple molecular scale scenarios. These scientific issues are also intimately linked to the biotechnological applications that mostly include immuno-diagnosis and cell sorting. It is remarkable that all of these single-molecule experiments performed with AFM, the red blood cell and vesicle force probe, the laminar flow chamber, and more recently with self-assembled magnetic chains, offer an important guide for designing more precise and sensitive diagnostic tools. Indeed, the information gained from each of the relevant molecular parameters controlling the rate and specificity of recognition directly impacts the efficiency of bio-analysis strategies. So far, it seems that quite a large contact or encounter duration is of critical importance for the recognition to be completed. This is certainly one of the more recurring conclusions throughout the recent literature, which is certainly opening up a new debate about the origin and quantification of this surprisingly large binding time. Probing and quantifying the variations of this time as a function of precisely controlled microscopic parameters, besides the nature of the ligand/receptor couple, will certainly be part of the next step.

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